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EXPERIMENTALLY INDUCED ROUND WINDOW MEMBRANE LESIONS

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(Received July 24 1976)

ract This investigation confirmed previous studies ating that the middle and inner ear of the guinea pig a pronounced tendency to healing Middle ear orrhage of surgical origin appears to stop quickly in guinea pig A surgically created perforation of the d window with or without removal of perilymphatic by suction resulted in a spontaneous healing of the d window membrane in 12/13 animals Perilymphatic orrhage, associated with short post-surgical interval found in a substantial number of cases The origin e perilymphatic hemorrhage is doubtful Degenera of the organ of Corti was found in four ears in three als and appeared to be correlated to the peri hatic hemorrhage Electrophysiological measure s appeared to correlate well with the anatomical ngs for one animal with very small changes and for animals with pronounced middle and inner ear ology In three additional animals less agreement was rved In general these animals showed a more pro- ced decrease of function than would be expected e anatomy

most reported cases of sudden deaf- the etiology has been obscure In part this ue to the more or less universal lack of ana ical and pathological information related to e cases This has led to much unsubstan d speculation However, recently one ested etiology was confirmed in several nts subjected to barotrauma, i e , round oval window rupture (Goodhill, 1971, , Pullen, 1972, Leek & Riess, 1973, Freeman, 1973, Goodhill et al , 1973, Azem & Caldarelli, 1973, Freeman et al , 1974, Fraser & Harborow, 1975, Sasaki et al , 1975, Taylor & Bicknell, 1976)

Aural symptoms in connection with diving and flying are well known The symptoms and signs have usually been limited to the middle ear space with varying degrees of pathology from slight injection of the tympanic mem brane to hemorrhage in the middle ear and rupture of the tympanic membrane Audio logic findings associated with such changes in the middle ear are usually restricted to air conducted changes in sensitivity The changes have, for the most part, been attributed to impaired tubal function with resultant inability to equalize the middle ear pressure However, in addition to these obvious cases of middle ear barotrauma, cases of sudden sensori neural deafness associated with sudden pressure changes in the middle ear have been reported These cases have not been ade quately explained Many of them have demonstrated improved hearing during subsequent weeks or months The most common presumed etiology has been inner ear hemorrhage which presumably cleared slowly The recently reported cases of oval and round window rupture offer an alternative etiology In this case, recovery may be associated with spontaneous healing and sealing of round and oval window membrane fistulas

research was supported by the Swedish Medical Re h Council (B76-12X-00558 12C) and Swedish board chnical development 75-4159 and Gotaverken AB by a contract from the Office of Naval Research 014 75-C-0463)

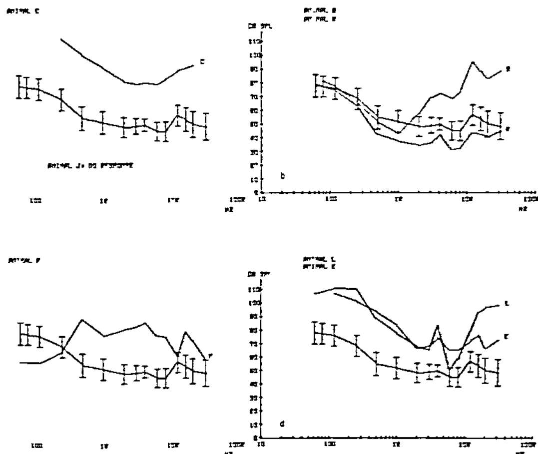
Simmons et al (1962) created round window perforations in the cat and followed the healing with behavioral audiometry as well as studies of cochlear microphonic activity Based upon cochlear microphonic measures they observed a loss of about 35 dB For most subjects recovery was relatively rapid and the microphonic response returned to within a few dB of preoperative levels by 1–4 weeks However, in about one-third of the ears, cochlear micro phonics did not return to preoperative levels This was presumed due either to technical failures, infection, or undisclosed causes Their behavioral findings were inconsistent with the microphonic studies in that they found essentially complete recovery (two animals) within a week On the basis of their observations of functional recovery the authors suggested that the fluid leak from the round window probably lasted less than one day

The aim of the present investigation was to provide additional information on the effects of round window membrane perforations on electrophysiological response of the cochlea, to obtain correlational observations concerning histological features of the inner ear structures associated with round window perfora-s and to further examine healing of the per-ed round window membrane

MATERIAL AND METHODS

Thirteen healthy adult guinea pigs with normal Preyer's reflex were used Under general anesthesia, the middle ear was opened using a post auricular approach After careful inspection of the middle ear, a slit was created in the round window membrane with a sharp micro knife The slit was made longer than half of the diameter of the round window Resultant perilymph effusion or hemorrhage was observed for five to ten minutes In six animals perilymph was subsequently sucked away with a resultant air bubble observed in the scala tympani of the basal turn The round window was left open and the middle ear bulla was closed The animals were not plac any drug regime Eight animals wer minated within two weeks after surger the remaining five between thirty si ninety-five days after surgery Electrop logical changes in the round window co microphonic (CM) activity were exami seven animals Just prior to sacrifice, th mals were intubated and respirated with thrane anesthesia (Lamkin & McPh 1975) The middle ear was again expos a post-auricular approach A platinum ı ball electrode was placed on the round w and sealed The round window was with dental cement and a closed sound s (TDH-39 speaker) was introduced int external auditory meatus Pure tone s generated by a beat frequency oscillator radiometer wave analyzer were used, int of the sound was measured with a calib probe tube and one-half inch Bruel and condensor microphone placed two millin lateral to the tympanic membrane CM p tials were amplified with standard el physiological equipment and displayed oscilloscope The amplitude of the CM recorded was measured with a General wave analyzer Pure tones employed ra from 60 Hz through 12 kHz The inte of each pure tone stimulus necessary to a one microvolt (rms) was determ Control observations from forty-three n guinea pigs were used to reference our ex mental findings The average of these co observations including + and −1 stan deviation is shown in Fig 1

Following the electrophysiological stu the animals were sacrificed, the temp bones removed and the bullas opened those animals not undergoing electroph logical study, sacrifice and temporal b removal was also performed after the sub was anesthetized The cochlea was asse according to procedures previously desc (Axelsson et al , 1974, 1975) Briefly cochlea was fixed by slowly injecting glutaraldehyde from an apical opening



1 Individual electrophysiological recordings from en guinea pigs subjected to previous mechanical round dow perforations Each figure also shows the average V CM±1 standard deviation from 43 normal guinea s (*a*) Two animals showing pronounced decreased response (C) and no response (J) (*b*) Animal B showing a decreasing CM response in the high frequencies and animal K showing normal response (*c*) Animal F showing a variable decrease in CM response in the middle and high frequencies (*d*) Two animals (E L) showing a loss of CM throughout the frequency range

m the oval window with the stapes re ved After 24 hours fixation the cochlea › decalcified in 5% EDTA with daily nges of solution until the cochlea was pletely decalcified (usually 7 days) An co-basal section was made of the cochlea l all structures carefully observed in the eo-microscope The cochlea was then nterstained in 1% osmic acid for 12 min, ydrated in a series of increasing concentra- s of alcohols, and transferred to glycerin er further dissection the cochlear speci s were examined with stereo- and phase trast microscopes

RESULTS

Surgery

Of the first seven animals (A–G) in which no perilymph was removed by suction four exhibited no perilymph leakage (A, C, D, G) and three (B, E, F) a small leakage for 15, 30 or 60 sec before it stopped spontaneously Minimal hemorrhage was observed in four animals (B, C, D, F) It stopped spontaneously within 30 sec

In the subsequent six animals (H–M) perilymph was removed by suction from the round window until a clearly visible air bubble could be observed on the inside of the membrane

Table I *Round window membrane lesions Histological findings*
o/c=operated/control

	Animal											
	A o/c	B o/c	C o/c	D o/c	E o/c	F o/c	G o/c	H o/c	I o/c	J o/c	K o/c	L o/
Post surgery period days	95	10	9	14	14	10	10	14	13	36	56	5:
Electrophysiology		+	+		+	+				+	+	+
Middle ear												
Otitis	+/		+/	+/+	/+		+/	+/		+/+		
Serous effusion		+/			+/	+/			+/			+
Hemorrhage		+/	+/+	+/		+/			+/			
Round window niche												
Perforation healed	+		+	+	+	+	+	+	+	+	+	+
Cochlea[a]												
Perilymphatic hemorrhage		*1–4/1*	*1–4/1–4*	*1–2/1*		*1/*	*/1*	*/1*		*1/*		
Strial degeneration	3/			1/						1/	4/	
OOC degeneration[b]		3–4/	1–2/1–2							1–4/		

[a] Figures indicate turn of cochlea with demonstrated changes
[b] OOC=Organ of Corti

Hemorrhage was observed in one of these six animals (I) It stopped spontaneously within approximately 30 sec In one animal (L) some purulent secretion was found in the middle ear during surgery The round window membrane was perforated and perilymph ucked away Interestingly after 52 days sur l time for this animal only some clear fluid found in the middle ear there was no sign itis media

Electrophysiology

As may be seen in Fig 1 a variety of changes in cochlear activity was observed The greatest change in cochlear activity was observed in animals C and J (Fig 1*a*) It was not possible at the highest intensity of stimulation to elicit a 1 μV CM potential for any frequency for animal J Animal C showed a relative flat elevated threshold of approximately 40 dB over a frequency range of 200 Hz to 11 kHz The least change was seen in animals B and K (Fig 1*b*) Animal B shows no change from normal for low frequency tones (through 1 000 Hz) with an increasingly elevated threshold change for frequencies over 1 000 Hz At the highest frequencies ined, the elevation amounts to 35– Animal K shows no change from i Animal F showed a relatively normal CM for low frequencies and a relativ loss of 30 dB throughout the middle and frequencies examined (Fig 1*c*) An (Fig 1*d*) exhibited a relatively flat loss 25 dB throughout the frequency range ined Animal L showed a similar flat l frequencies up to approximately 4 kHz ever, above that level it showed a son inconsistent elevation in threshold (Fi

Histology

Middle ear (see Table I for tabulation of these findings)

A frequent finding was otitis media Th found bilaterally in three animals (D on the operated side only in four a (A C G H) and on the contralateral operated side in one animal (E) Othe mon findings were serous effusio hemorrhage in the middle ear on the op side This leaves only one animal with pletely clear middle ears (K) When the



Guinea pig (Animal B) Round window 10 days mechanical perforation with a remaining peri lymphatic fistula Perilymphatic hemorrhage (arrows) is seen on the inside of the round window membrane

indings were correlated to the post interval it was found that otitis and of secretion or hemorrhage in the ear were more common in the eight with a short post-surgical interval) than in the five animals with a long survival time After a short interval 6/16 ears demonstrated otitis 4/16 serous secretion and 6/16 hemorrhage After a long post-surgical interval 5/10 ears had otitis, 1/10 serous secretion and 0/10 hemorrhage Consequently, it appeared that serous secretion and hemor



Fg 2b The perforation n higher magnifcat on In tial heal ng is ind cated by a fb ous strand across the per foration

rhage in the middle ear induced by middle ear surgery may heal spontaneously with time

Round window membrane n che (see Table I) Hemorrhage on the outside of the round window membrane as well as n the middle ear was observed in five animals all of whi belong to the group which was assessed w th two weeks (B C D F I) In one of the animals the contralateral ear also show hemorrhage in the middle ear and on the rou window membrane (C)

ne perforation of the round window was 'ed in all cases but one in which there ıained two small perforations divided by a scar (Animal B, Fig 2) The post-surgical ı in this animal was among the shortest, ys In most other cases not even scars visible on the round window membrane observed in low magnification with the ˀ microscope

' (see Table I)

˯ in the scala tympani was ob-ᵉᵈ bilaterally in three animals (B, C, D), operated side only in two animals (F, J) on the control ear only in two animals H ı (Figs 3, 4) All these animals, except (J) belong to the group with a short post-ıcal interval One of these animals showed fibrotic development of the hemorrhage scala tympani on the operated ear (J, ˗˯ survival time) (Fig 4) Hemorrhage perilymph of the scala vestibuli was in four animals (B, C, D, J) who all hemorrhage in the scala tympani as

only ear with a remaining round win-perforation (Animal B) also exhibited pro-changes in the cochlea with hemor-both in the scala tympani (Fig 2) and vestibuli and apical degeneration of the of Corti (OOC) The basal turn did not OOC-changes

eration of the stria vascularis was ıd in *four animals on the operated ear*), J, K)

˗ ɲn of OOC was found in three ˢ (B, C, J) In one of these animals the were found on both ears and in the regions of both cochleas (C) Vessels supporting structures such as the spiral the spiral limbus and supporting in all subjects appeared normal bilat-ɏ

general, we note (i) Degeneration of was more common in the operated ear ᵉneration of stria vascularis was more with a long post surgical survival time (iii) Middle ear and perilymphatic hemorrhage was more common with a short post-surgical survival interval

Degeneration of OOC was always asso ciated with perilymphatic hemorrhage In half of the ears with middle ear hemorrhage there was a degeneration of OOC, in 3/4 of the ears with degeneration of the OOC, a middle ear hemorrhage was found

No correlation was observed between (i) animals with short or long post-surgical interval and the occurrence of otitis media or degeneration of OOC (ii) ears with otitis media or strial degeneration and other changes

In regard to the electrophysiological findings The observations of basal turn OOC degeneration was clearly correlated with a decrease in CM sensitivity Similarly, perilymphatic hemorrhage of the first turn was associated with a reduced CM output

DISCUSSION

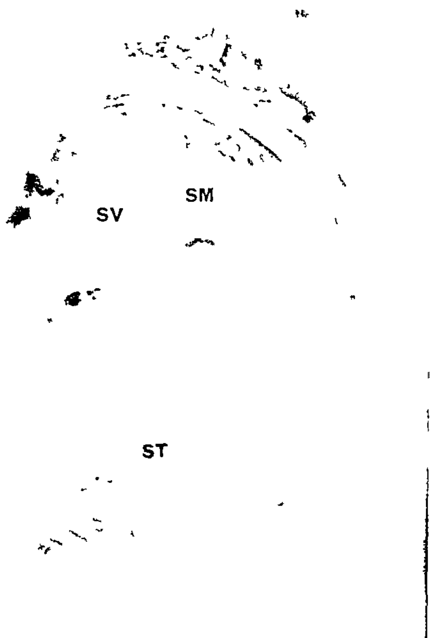
The most interesting finding of the present investigation is the histological observation of spontaneous healing of the guinea pig round window after trauma This confirms our pre vious results (Axelsson & Hallen, 1973, Hallen et al , 1974*a*, *b*) Only in one animal out of thirteen did the mechanically induced perforation of the round window membrane not heal The healing was achieved in spite of a fairly frequent otitis media occurrence of serous *secretion and hemorrhage after surgery* *These* and previous observations suggest that the guinea pig cochlea has a pronounced tendency to heal after a variety of mechanical traumas

The pronounced healing tendency of perforations on the guinea pig round window could be used to argue that human cases with round window fistulas do not necessarily need surgical repair, however, in the previously published cases there is a clear correlation between the interval of the onset of the peri lymphatic fistula and the subsequent remaining sensorineural hearing loss The longer the interval, the greater the hearing loss



Fig 3 Guinea pig (Animal F) basal turn longitudinal section The most common cochlear finding after round window perforation was hemorrhage (arrows) in the tympani (*ST*) *SV*=scala vestibuli *SM*=scala med a

(Goodhill, 1971, 1973 Pullen 1972 Freeman 1973, Azem & Caldarelli 1973 Fraser & Harborow, 1975, Sasaki et al 1975) This clearly indicates that surgical measures to repair the round window perforation should not be delayed too long In the present investigat it appears that most perforations in the gu pig will heal within two weeks However do not know the minimum time for suc spontaneous healing Neither do we kno



Guinea pig (Animal J) basal turn longitudinal A small hemorrhage is seen in the scala vestibuli (*SV*) The only animal with a massive hemorrhage of The stria vascularis (*SVS*) is detached from the external tympani (*ST*) with initial fibrotic development wall *SM* = scala media

man round window has the same healing as the guinea pig

occurrence of intracochlear hemor is difficult to explain During surgery hemorrhage was observed and this stopped shortly and spontaneously Such hemorrhage may result from the surgical intervention the middle ear approach for our electrophysiological studies or the mode of termination of the animal and the maneuvers

employed durıng temporal bone removal which may produce an elevated venous pressure Only ın one case ın the present material was there a clear ındication of an old hemorrhage with a beginning organization of the hemorrhage to fibrosis

Strial degeneration was found in four operated ears both basally and apically and was in these cases markedly different from the control ear We have no explanation for the degeneration of the stria vascularis which was found in 3/5 animals after long post-surgical intervals The degeneration of the stria vascularis did not correlate with any other findings, histological or electrophysiological

Degeneration of the OOC was found in four ears in three animals and was more common basally than apically Surprisingly, the animal with the remaining round window perforation (B) demonstrated a degeneration of OOC apically and intact OOC basally Perilymphatic hemorrhage was found throughout this cochlea In two other animals (C, J) pro- degeneration of OOC in the basal was observed along with perilymphatic -age

- ear changes such as otitis, serous or purulent otitis and hemorrhage were more common in animals with a short post surgical interval This would be expected with a heal ing tendency of the middle ear and clearing up of such changes with time Of interest was the animal which exhibited otitis during surgery and in which the middle ear appeared normal on the subsequent assessment

It is noted that a variety of changes were seen in the electrophysiological response of the cochlea observed in these seven animals following round window perforation Changes range from a normal response (Animal K) to complete lack of cochlear activity (Animal J) Most of the electrophysiological changes can be accounted for by the changes seen in the cochlea, with perhaps some contribution from middle ear changes In animal J which ex hibited no electrophysiological response de generation of the OOC was seen fr m the basal turn throughout the cochlea This combined with strial degeneration in the b turn, longstanding perilymphatic hemorrh in the basal turn, and indication of otitis in middle ear The electrophysiological findi are certainly most consistent with these a tomical observations For animal C which hibited a flat loss of approximately 40 dE degeneration of OOC was observed in basal and second turns of the cochlea and p lymphatic hemorrhage throughout the cc lea With the round window as the record site for the cochlear microphonic, we wo not expect a very great contribution fr remote hair cells, i e those beyond the f turn of the cochlea

In animal K the normal electrophysiologi findings are also consistent with the ana mical observations Animal B exhibited normal response for low frequency tones a a sloping increasing loss for higher frequ cies Clear OOC degeneration was restric to apical parts of the cochlea We would pect the apical degeneration to have li influence on the electrophysiological observ tions However, we would expect basal tu perilymphatic hemorrhage and a perilympha leak through the round window perforation contribute to the decreased CM recordings

For animal F no cochlear degeneration w observed Hemorrhage in the basal turn and the middle ear may account for the electr physiological findings of more or less general decreased CM recordings However, suc changes in the middle ear, coupled with per lymphatic hemorrhage were also observed animal B in which the electrophysiologic changes were restricted to the high frequen cies

The electrophysiological results on animal E and L did not correlate well with the ana tomical findings Both animals demonstrated decreased overall CM response The flat los observed for animal E may be secondary t the effects of otitis observed in this animal However, only minor changes were observed in the middle and inner ear

In summary, we note that for the three animals with pronounced anatomical changes (B, C, J) as well as for one more or less 'normal' animal (K) a clear correlation to electrophysiological measurements could be observed. The variable and somewhat smaller changes observed in animals E, F and particularly L cannot be explained well on the basis of our anatomical observations.

ZUSAMMENFASSUNG

Die Untersuchung bestätigte die Resultate fruherer Stu

Meerschweinchen schnell zum Stehen zu kommen. Eine chirurgisch hervorgerufene Perforation des runden Fen

Blutung in einer bedeutenden Zahl von Fällen gefunden. Der Ursprung der perilymphatischen Blutung ist zweifelhaft. Degeneration des Cortischen Organes wurde in vier Ohren von drei Tieren gefunden und schien mit der perilymphatischen Blutung zu korrelieren. Elektrophysiologische Messungen schienen gut zu korrelieren mit den anatomischen Befunden bei einem Tier mit leichten Veränderungen und bei drei Tieren mit ausgesprochener Mittel- und Innenohrpathologie. In drei weiteren Tieren war die Ubereinstimmung weniger augenscheinlich. Im allgemeinen zeigten diese Tiere ein starkeres Abnehmen der Funktion als aus der Anatomie zu erwarten war.

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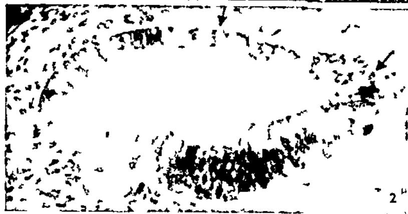
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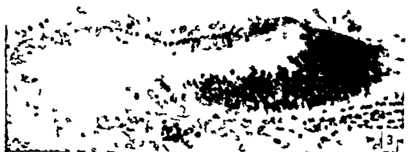
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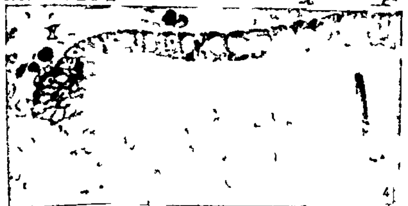
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Sweden









F gs 1–8 Light m crographs
F g 1 Fourteen-day embryo m ear Some of the ep thel al cells stained more darkly than others the d stribution of the darker ce d d not coinc de w th that of gly cogen particles as judged by ele tron m croscopy (10× objectı e)
F g 2 E ghteen-day rat embryo The dark sta n ng of the cells of future Reissner s membrane (be tween arrows) was revealed to b due to glycogen particles when e am ned with the electron m cro-scope (16× objective)
F g 3 In a 19-day embryo glyco gen is found in the future pillar c as well as in Re ssner's membran (16× objective)
F g 4 H gher magnif cation view of another 19-day embryo to dem onstrate dark staining of Re ss-ner's membrane and future p llar cells (40× o l objective)





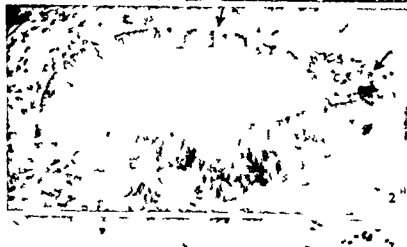
Fig 5 Diastase treatment removed the material that stained darkly in control specimens helping to demonstrate that it was glycogen Twenty two-day embryo showing cleared area in future pillar cells (16× objective)

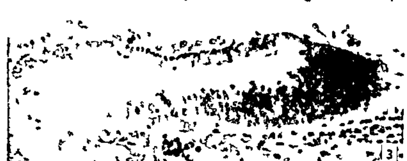
Fig 6 At the newborn stage the cells of the future stria vascularis stain unevenly with this technique but the darker cells did not necessarily contain more glycogen than the lighter ones when examined with the electron microscope (16× objective)

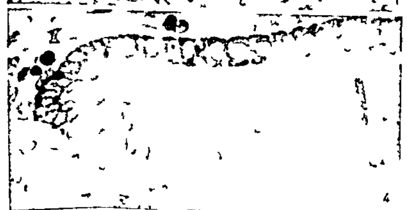
Fig 7 Two layers of dark material appeared in the stria vascularis by 3 days after birth Note persistence of stain in the future pillar cells (16× objective)

Fig 8 By 10 days after birth no glycogen is evident with this technique in any of the cells of the cochlear duct by light microscopy (16× objective)





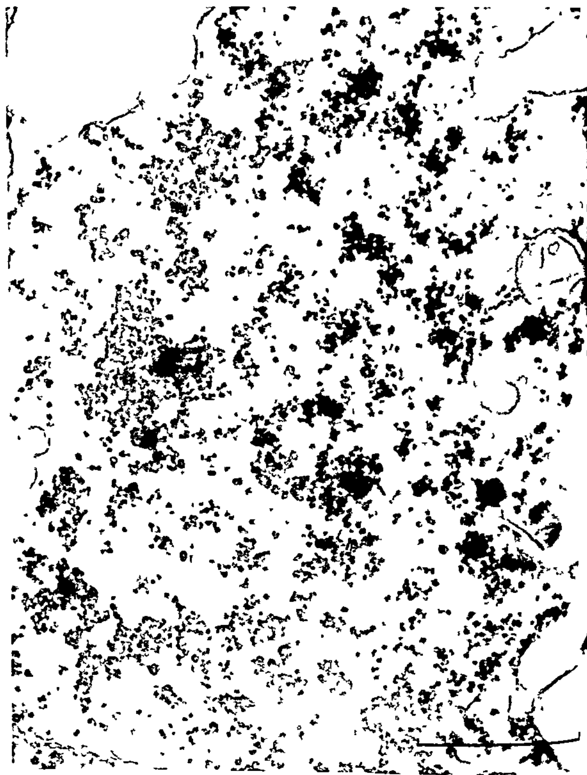




F gs 1–8 Lıght mıcrographs
F g 1 Fourteen-day embryo innı ear Some of the epıthel al cells staıned more darkly than others t the d strıbutıon of the darker cells d d not coınc de wıth that of gly cogen partıcles as judged by elec tron m croscopy (10× objectıve)
F g 2 E ghteen-day rat embryo The dark sta n ng of the cells of future Re ssner's membrane (be tween arrows) was revealed to be due to glycogen partıcles when ex am ned w th the electron m croscope (16× objectıve)
F g 3 In a 19-day embryo glycogen s found ın the future p llar cell as well as ın Re ssner s membrane (16× objectıve)
F g 4 H gher magnıf catıon v ew of another 19-day embryo to demonstrate dark staınıng of Re ss ner s membrane and future pıllar cells (40× o l objectıve)



Fig 10 Seventeen-day rat embryo Reissner's membrane with heavy deposits of glycogen clumps in the epithelial cells



Figs 9–14 Electron micrographs
Fig 9 Fourteen-day embryo inner ear epithelial cell with glycogen particles distributed fairly evenly in cytoplasm This dispersed glycogen was not visible by light microscopy (Marker indicates 1 μm)

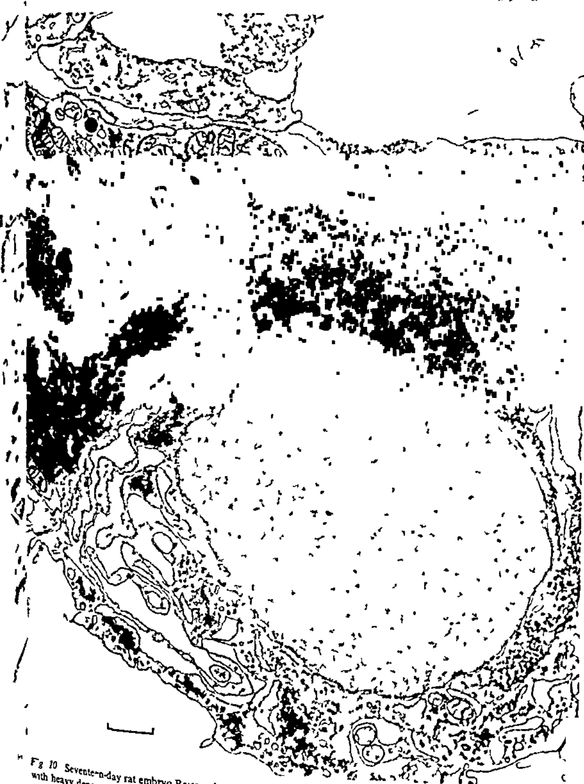


Fig 10 Seventeen-day rat embryo Reissner's membrane with heavy deposits of glycogen *clumps* in the epithelial cells



Fig. 11. Twenty-one-day rat embryo marginal cell from the stria vascularis. At this stage the glycogen is too dispersed to be obvious by light microscopy but the ules are obvious by electron microscopy in the stria



Fig 12 Twenty two-day embryo showing glycogen deposits in pillar cells Inset is light micrograph (40× objective) showing Köllikers organ (*left*) and future outer hair cells (*right*) of the dark [illegible] pillar cells (*P*) in the el[illegible] amounts of glycogen

hair cells by electron microscopy, but light microscopy showed no evidence of staining In the adult, outer hair cells of all turns showed about the same concentration No glycogen is evident in [illegible] other tissue of the [illegible]

Fig 13 is an ele[illegible] vascularis of a ne[illegible]



Fig 13 A variety of artefacts result from the interaction of glycogen with the reagents used for processing tissue unless potassium ferrocyanide is used with uranyl acetate to preserve the glycogen. Myelin figures probably represent clumped glycogen. "Moth eaten" defects in cytoplasm of marginal cells resulted from leaching of the glycogen.



g 14 Stria vascularis By 3 days after birth heavy gly 'gen deposits are obvious in the stria vascularis by light and electron microscopy Inset is light micrograph (40× objective)

lustrates the artefacts that are prevented by adding potassium ferrocyanide to osmium tetroxide during post fixation This specimen was treated the same as the others, except for this step 'Moth-eaten' areas in the cytoplasm occurred because glycogen had been leached out during processing Close examination of the illustration will show differences in density within these areas which can be explained by assuming that the glycogen was partly removed during processing of the block of tissue before embedding and more was later removed from the plastic thin section by the water of the trough or of the staining solution "Myelin-figures" and dense, irregular shaped bodies were probably the result of an interaction between glycogen in the tissue and various reagents

DISCUSSION

According to recent studies, glycogen can be reliably localized in tissue by electron microscopy if an appropriately modified osmium reagent is used after initial aldehyde fixation (De Bruijn, 1973, Fawcett & Dym, 1974) We used this technique to study distribution of glycogen in the cochlear duct during several of development

light microscopic results agreed with Hansen's (1967) However, subsequent microscopic examination of the speci mens led us to interpret light microscopic findings somewhat differently We learned that glycogen was dispersed in the cytoplasm of all the otocyst cells with no major differences between cells that would coincide with light microscopic variations

Reissner's membrane is the first portion of the cochlear duct to show heavy deposits of glycogen By the stage of the 18 day embryo, it is evident by light and by electron micro scopy Earlier, glycogen had been evenly distributed through the future stria, the future promontory and Reissner s membrane, an electron microscopic finding not evident with this technique by light microscopy

Future pillar cells soon afterward (19-day embryo) acquire heavy deposits of glycog obvious by both light and electron mic scopy, and the stria epithelium shows s nificant quantities shortly before birth

Glycogen stored in Reissner's membrane the stria vascularis could be expected to b source of energy for fluid transport In each these tissues, it appears at a key stage Reissner's during the appearance of scala v tibuli and in the stria during its conversion t three layered complex The pillar cells cont glycogen prior to the formation of the tunnel Corti One wonders if active fluid transpor involved in forming the tunnel space

Falbe-Hansen's (1963) histochemical stud suggested that apical turn outer hair cells co tain more glycogen than those of the ba turn Microchemical analysis has also sho more glycogen in the upper turns of the org of Corti than in the basal portion (Matchinsl 1970, Thalmann, 1975) With electron mic scopy we found roughly equal concentratio of finely dispersed glycogen granules in ou hair cells, regardless of which turn

The hair cell size difference may be the e planation for the greater quantity of glycog in apical as opposed to basal turn organ Corti Of course, our judgement with respe to concentration is subject to the limitations any morphological method for the estimati of chemical content

ZUSAMMENFASSUNG

Eine neue Methode mit Licht und Elektronenmil skopie wurde angewandt um Glykogen in der Schne der Ratte im Entwicklungsstadium zu beobachten dem vierzehntagigen Embryo ist das Glykogen feina im ganzen inneren Ohr verbreitet Die ersten gro Niederschläge wurden in der Reißnerschen Membr beobachtet dann in den Saulenzellen Diese verschw den bei der Geburtszeit als große Klumpen von Gly gen *in der Stria vascularis gefunden wurden* In aus wachsenen Tieren haben die außeren Haarzellen sic bare Glykogen welches feinartig verbreitet ist

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PIGMENTATION OF THE STRIA VASCULARIS

The Contribution of Neural Crest Melanocytes

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Abstract Because all stages of melanogenesis (premel anosome melanosome and melanin granules) were found in intermediate cells of the rat stria vascularis they can be classified as melanocytes The marginal and basal cells of these rat specimens contained no p gment Early in development the melanocytes or future intermediate cells are located beneath the strial basal lamina They penetrate this lamina to insert themselves between mar ginal cells Both the melanocytes and marginal cells are believed to participate actively in the tissue rearrange ments which ultimately bring the two cell types into in timate structural relationship The ingrowing melanocytes and the mature pigmented intermediate cells which they form are frequently associated with blood vessels Pre treatment with Dopa to enhance the melanin content of early melanocytes d d not enable us to identify their route m gration into the stria

) process of melanosome formation in vari üs tissues has been described by several in vestigators (Seiji et al 1961, Birbeck 1963, Seiji 1967) Initially vesicles appear in the cytoplasm Later fine fibrillar material is found in these vesicles As the process con tinues, the fibrillar material becomes cross striated and may take the form of lamellae Melanin deposition occurs on this matrix As more melanin is added the fine fibrils thicken lose their cross striation and then become ropy strands (Jimbow & Kukita 1970) The initial vesicles are thought to bud off s surfaced endoplasmic reticulum Incu with tritiated pressurers (Dopa, tyrosin examination by radioautography show corporation of labeled substances close smooth surfaced endoplasmic reticulu support of this hypothesis (Zelickson 1964) Other evidence suggests that sinase, one enzyme responsible for m formation is synthesized by ribosome transferred to melanosomes where mela deposited (Toda & Fitzpatrick, 1970)

Seiji et al (1961) introduced the term melanosome and melanosome to descrit immature melanin forming organelles Z son (1967) summarizes the terminolog these organelles during melanogenesis premelanosome is thus defined as an org of varying shape and size, produced melanocyte and consisting of a protein n of cross linked fibers with a 90 Å stri upon which melanin may or may not b posited A melanosome would then be de as a premelanosome upon which melan been deposited The amount of melanin size and shape of the granule, all of w determine the final form of the mature me granule will vary depending on various g ic factors Seiji (1961) defined melanoso the enzymatically active stage that prec the mature melanin granule which lacks sinase It is generally agreed that the mel

A major portion of this invest gation was performed at the Section of Otolaryngology New Jersey Med cal School Newark New Jersey

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orming cells, melanocytes, are neural crest derivatives (Weston, 1970)

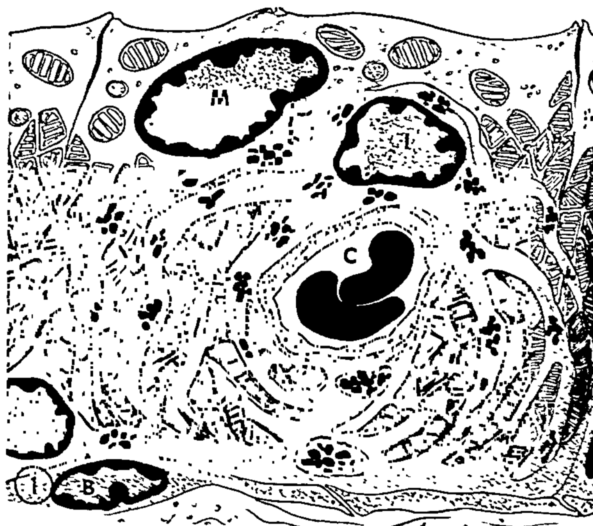
The stria vascularis is a unique epithelium because it contains capillaries, is composed of cells from more than a single embryological source, and has no basal lamina beneath it Three types of cell have been identified in the stria marginal, intermediate and basal (Rodriguez Echandia & Burgos, 1965, Hinojosa & Rodríguez-Echandia, 1966) Marginal cells line the endolymphatic surface Their cytoplasm is packed with organelles and their plasmalemmae are extensively folded giving them a dense appearance Intermediate cells are much lighter and are less densely packed with organelles and plasmalemmal infoldings Basal cells contain the fewest organelles Evidence was found by Kikuchi & Hilding (1972) suggesting that cells from different embryonic sources contribute to the formation of the stria In mice they observed cells from the spiral ligament combining with marginal cells of otocyst epithelium derivation to form the stria In the process the basal lamina, which is characteristically found beneath epithelia, disappeared Later Hilding (1969) found ' migrating cells ' in mink cochleas that seemed to enter into formation of the stria

Pigmentation has been recognized in the cochlea since the time of Corti (Savin, 1965), and more recently pigment granules in the stria have been demonstrated by means of the electron microscope (Smith, 1957, Rodriguez Echandia & Burgos, 1965, Hinojosa & Rodríguez Echandia, 1966) In the human, pigment cells are found in Reissner's membrane, near the modiolus and on the vessel of the spiral prominence as well as in the stria vascularis (La Ferriere et al , 1974) In most other mammals cochlear pigment seems to be mainly confined to the stria vascularis

MATERIALS AND METHODS

Adult, juvenile and fetal Long Evans rats a pigmented strain, and Sprague Dawley albino rats were examined The animals were sacrificed at varying ages adult rats at 63 days old, juveniles at 16, 12, 9, 4 days old and new born to give a sampling of the developmental and mature states Embryos of 17, 18 and 19 days gestation staged by length and weight and plug dating of the mothers were also examined The mother rats and the other adult rats were anesthetized with Nembutal (3 mg per 100 g body weight), while the younger animals including the embryos were decapitated before dissection of the cochleas As soon as possible after the dissection was begun, fixative was perfused through the round and oval windows of the cochleas The primary fixative consisted of 2% para formaldehyde and 2 5% ultrapure glutaraldehyde dissolved in 0 08 M cacodylate buffer at pH 7 4 with 0 05% calcium chloride The dissected cochleas were fixed at room temperature and stored in fixative at 4°C overnight The following day they were rinsed briefly in 0 08 M cacodylate buffer with 0 18 M sucrose and 0 05% calcium chloride and post fixed in 2% osmium tetroxide dissolved in the above buffer for 1 or 2 hours They were then rinsed in 0 05 M maleic buffer and stained at 4° Centigrade in maleic buffered 2% uranyl acetate at pH 5 0 After dehydration the cochleas were embedded without further dissection in a hard (2 1) Epon mixture Two of the embryonic specimens (17 and 19 days embryos) were immersed in a primary fixative containing 4% para formaldehyde and 5% glutaraldehyde with good results

The Epon-embedded cochleas were cracked in a plane as close to mid modiolar as possible and the areas of interest were selected after thick sectioning Thin (600–900 Å) sections were collected on unsupported or Formvar-coated grids The adult specimens were stained 10 min in 2% uranyl acetate dissolved in 100% methanol and 10 min in Reynolds lead citrate (2 66%) The other specimens were stained 60 sec in 2% uranyl acetate in 50% ethanol and 15 sec in 0 1% lead citrate Specimens were examined with a Philips 300 electron microscope



This diagram illustrates the octopus-like form of cells (*I*), which tend to "sit" on blood ves- C) and send tentacle-like dendrites between folded marginal cell processes. Basal cells (*B*) form a flat she beneath intermediate and marginal cells (*M*)

Tyrosinase reaction

Specimens to be incubated in Dopa to enhance melanin deposition were immersed for 1 hour in a fixative containing 2% formaldehyde and 2.5% glutaraldehyde dissolved in 0.1 M phosphate buffer at pH 7.4. After a brief rinse in phosphate buffer the cochleas were incubated for approximately 18 hours at 37°C in 1% L-β-3,4-dihydroxyphenylalanine (L-Dopa) dissolved in phosphate buffer as suggested by Becker (1942) and Mishima et al. (1962) and Mishima (1964). After incubation and a brief rinse in phosphate buffer the cochleas were post-fixed with 2% osmium tetroxide in phosphate buffer and dehydrated. *En bloc* stain with 1% phosphotungstic acid dissolved i absolute alcohol for 1 hour was the final st before embedding (Mishima et al., 1962). The sections cut from such specimens were stained as noted above in uranyl acetate and lea citrate.

RESULTS

The normal adult stria vascularis is composed of three cell types, as shown diagrammatically in Fig. 1. Marginal cells line the endolym phatic space and are the most darkly stain



Fig 2. Adult rat stria vascularis, survey electron micrograph. Pigmented intermediate cell and cell processes (*I*) intimately relate to marginal cell outfoldings. A capillary (*C*) has a generous area of contact with an intermediate cell. *M*, marginal cell, *B*, basal cell, *P* melanin pigment. Original magnification, ×3300. (Magnification figures refer to original 8.3×10.2 cm electron micrograph negative. The figures published are enlarged approximately twice.)



Fig 7 Eleven-day albino rat showing cell organelles which may represent non melanized premelanosome (*arrows*) The fibrillar material has a well-ordered crystaline appearance *L* lipid droplet *M* marginal cell ×16 000



8 Nineteen-day embryo A cell process from a mel- (P) appears to be entering between two marginal ls (M) through a defect in the basal lamina Glycogen occupied the "moth-eaten" appearing portions of the marginal cells (*arrows*) ×10 000

ig the subsequent days of embryonic de- lopment, more and more melanocytes with ingly numerous and mature pigment s are found in the spaces between mar- nal cells Their processes increasingly inter- gitate with those of the marginal cells, their become located closer to the epithelial order, and they occupy an increased area of stria vascularis (Fig 3) The basal lamina hich underlined the strial epithelium in the oungest animals becomes increasingly frag- ented as more cells enter the stria and eventually disappears altogether, leaving only the basal laminae which surround strial capillaries

During a brief period in the development of the stria large amounts of glycogen are stored in the cytoplasm of the marginal cells No special steps were taken to preserve the glycogen in the present study We observed areas of the marginal cell cytoplasm which were devoid of organelles and had a "moth eaten" appearance (Figs 8, 9, arrows) Marginal cells were shown to contain glycogen in cc

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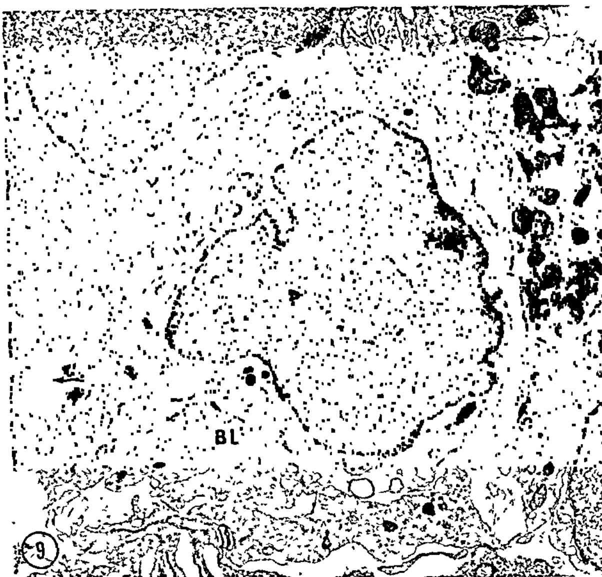


Fig 9 Nineteen-day embryo with melanocyte (*P*) enveloped by marginal cell processes The marginal cell basal lamina (*BL*) is absent in this area Glycogen was leached out of the marginal cells in this section to leav a moth-eaten appearance (*arrow*) ×6800

sponding areas in an earlier study of the rat stria (Hilding et al , 1977) The blotchy appearance is due to defects in the plastic which we interpret as a result of glycogen having been leached out of the sections as they floated on the water in the trough during sectioning.

In one series of animals the staining of melanocytes was enhanced by incubation with the melanin precursor Dopa Reaction product was noted in profiles of smooth-surfaced endoplasmic reticulum near the Golgi apparatus a others have reported (Endo & Hu, 1973 Zelickson et al , 1964) This histochemica technique should permit the observation o melanocytes *en route* to the stria in youn specimens The only stained melanocyte located at the ages studied here were in th connective tissue of the spiral ligament im mediately below the marginal cells and in th stria itself

DISCUSSION

ach of the stria's three cell types has distinc ve cytoplasmic features which make it pos ible to identify even isolated cell processes Marginal cells are rich in mitochondria, ribo omes and have a fine granular matrix Intermediate cells contain pigment in various stages of melanogenesis, scattered mitochondria ribosomes and both rough and smooth surfaced endoplasmic reticulum Basal cells con ain fibrillar material, have few mitochondria and ordinarily do not have ascending proces ses that enter the stria This classification would lead to somewhat different interpreta tions of cell types than have been made in the past (Smith, 1957, Rodriguez Echandia & Burgos, 1965, Hinojosa & Rodriguez-Echandia 1966)

Any cell that actively produces melanin is a melanocyte, *according to recent* concepts (Drochmans, 1963, Della Porta & Muhlbock, 1966) Our finding of all stages of melano genesis in the cytoplasm of intermediate cells means that they are melanocytes The intermediate cells are not believed to be "host" cells of melanin granules formed by other cells *since all* stages of melanogenesis were found in the intermediate cells When pigment is found in other types of "host cells these organelles show degradation (Drochmans, 1963)

Melanocytes are found associated with se cretorial epithelial cells in other portions of the inner ear (Kimura, 1969) LaFerriere et al (1974) showed in the vestibular labyrinth of guinea pigs that the melanocytes are usually closely associated with capillaries and capillaries are often extensively contacted by melanocyte processes The observed associa tion of melanocytes with blood vessels and capillaries in previous studies is supported in the present study, where melanocytes and their processes in both immature and mature rats frequently abut capillaries The migration of the melanocytes toward the stria during embryonic development could take place along blood vessels, although this hypothesis cannot be supported or denied on the basis of existing evidence

During the incorporation of melanocytes into the stria, two types of cell movement may be involved First, melanocyte processes may penetrate the basal lamina and the subsequent translocation of their nuclei through such *processes toward the epithelial border would* partially account for the mature configuration Second, the marginal cell processes may en velop capillaries and their associated melano cytes, thus accounting for the steadily increasing thickness of the stria during succeeding stages of development and the disappearance of the basal lamina beneath the marginal cells During these stages of maturation, the melano cytes send octopus like tentacles throughout the epithelium and establish an intimate relationship that is structurally quite unlike the situation in the vestibular labyrinth, where melanocytes are separated from the dark cells by generous extracellular fluid spaces and a basal lamina As LaFerriere et al (1974) also pointed out, the synthesis of melanin by melanosomes involves compounds of neuro secretory importance Perhaps the synthesis or release of such compounds is the basis of their function in the stria

The melanocytes of the stria closely resemble those in other locations such as skin and hair follicle All stages of melanogenesis can be observed in their dendritic processes as well as near the nucleus, although adult specimens showed a preponderance of more mature granules as compared with those from younger animals Albino animals had small, non pigmented crystalline organelles in inter mediate cells which resemble the unpigmented albino melanocytes that have been described elsewhere (Zelickson, 1967) As had been noted by Moellmann et al (1973), the crystal line organization of these albino premelano somes is particularly apparent because it is not obscured by dense melanin It appears likely, therefore, that the same cell type comprises the intermediate cells of albino animals and although through some genetic defect these

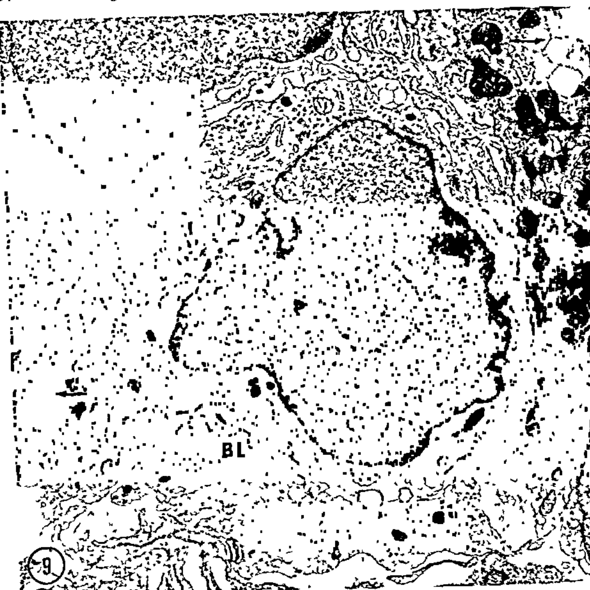


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DISCUSSION

Each of the stria's three cell types has distinctive cytoplasmic features which make it possible to identify even isolated cell processes Marginal cells are rich in mitochondria, ribosomes and have a fine granular matrix Intermediate cells contain pigment in various stages of melanogenesis, scattered mitochondria, ribosomes and both rough and smooth surfaced endoplasmic reticulum Basal cells contain fibrillar material, have few mitochondria and ordinarily do not have ascending processes that enter the stria This classification would lead to somewhat different interpretations of cell types than have been made in the past (Smith, 1957, Rodriguez-Echandia & Burgos, 1965, Hinojosa & Rodriguez Echandia, 1966)

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cells cannot synthesize melanin they are nevertheless of the same embryonic origin as the melanocyte intermediate cells of the pig mented strain

"Electron dense bodies of melanin appear ance" have been described in marginal cells (Rodriguez-Echandia & Burgos, 1965) Al though our rat marginal cells had none, it might be possible for marginal cells of other animals to act as "host cells" and contain ma ture melanin granules donated to them by mel- anocytes In that case, one would not expect to find various stages of melanogenesis in the host cell but instead would see mature and degraded granules

The association of pigment abnormalities in hair, eye and skin with cochlear deficit in hu- mans and various other mammals has led others to suspect an underlying defect of neural crest derivatives (Brown et al , 1971) Melanocytes, as judged by the results of this investigation, would appear to play an im- portant role in the development of the stria vascularis and comprise a major portion of ' tissue in the adult The absence of these , as a result of incomplete development of neural crest derivatives would likely to result in a loss of strial function, and could therefore be the basis of certain hearing losses as well It would seem possible that when stria from a patient with a combined pigment abnormality and hearing loss, such as Waardenberg s syndrome, becomes available for electron microscopy, abnormalities of the intermediate cell layer will be found

SUMMARY AND CONCLUSIONS

The process of pigmentation of the stria vas cularis was studied in a series of rats of vary ing ages, from 17-day embryo to adult Mel- anocytes which stained with Dopa were first sighted in the connective tissue of the spiral ligament They later insinuated themselves be tween marginal cells in the future stria and finally were found to have the characteristic shape and location of intermediate cells Both the marginal cells and the ingrowing pig mented intermediate cells or melanocytes ap peared to be actively involved in the tissu readjustments leading to the mature stru structure

Early melanocytes contained fewer mel anosomes than the more mature ones, bu mature intermediate cells contain all stages o melanogenesis, thus fulfilling the definition o melanocyte Furthermore, evidence wa found that the same cell type forms the inter mediate cells of the albino rat

It can be assumed that the intermediat cells, like other melanocytes, are derived fron the neural crest We have been unable t locate their route of migration into the spira ligament While the origin of the marginal and intermediate cells appears developmentall divergent, their intimate structural relation ship in the adult stria suggests that they are functionally interdependent The origin and function of the basal cells remains unclear, bu their location and apparently less intimat association with the other cell types of th stria suggests that they may be functionall separate as well

ZUSAMMENFASSUNG

Stria vascularis Zwischenzellen sind auch Pigmentzellen da man die Pigmentproduktion in diesen klar beobachten kann Diese Zellen befinden sich wahrend Entwicklung zwischen den Grenzenzellen (marginal cells) und pene trieren durch die Membrana basilaris

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VOLUME-PRESSURE PROPERTIES OF ROUND AND OVAL WINDOWS

A Quantitative Study on Human Temporal Bone

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(Received September 17, 1976)

Abstract A model study was carried out on human temporal bones to assess quantitatively the displacement of the round and oval windows when different pressure levels were applied to the external ear canal and inside the middle ear It was found that the major displacements of the window membranes occurred in the pressure range of ± 20 cm H_2O in the intact middle ear An additional increase in the middle ear pressure up to ± 70 cm H_2O caused a minor displacement of the window membranes

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It was also found that, considering the mobility of the windows the round window alone caused a 2–3 times greater displacement of perilymph than did the oval one

It seems urgent to assess and if possible, to quantify the extent of pressure transmission via the oval and round windows The purpose of continuing experiments will be to assess th[e] magnitude of a pressure transfer which woul[d] be great enough to impair the function of th[e] inner ear

The present investigation is a logical follow up of the clinical experiments that have bee[n] performed in this department over severa[l] years These experiments have indicated tha[t] a relative overpressure in the middle ear prob ably affects the inner ear function and, unde[r] certain circumstances, causes vertigo, so called alternobar vertigo (Ingelstedt et al 1974) Further, according to preliminary ex periments the relative overpressure seems t[o] relieve a Meniere's attack quickly in man[y] cases (Densert et al , 1975, Ingelstedt et al 1976) Thus, the aim of this study was to pro vide quantitative evaluation of the displace ment of the round and oval windows at diffe rent pressure levels applied to the external ea[r] canal and inside the middle ear

MATERIAL AND METHODS

Eleven human temporal bones were take[n] from 5 males and 6 females aged between 5[?] and 90 Clinical records showed no previou[s] otological disease The tympanic membrane[s] and the middle ears were examined under a[n]

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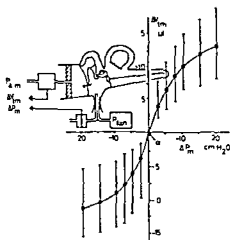


Fig 1 The displacements of the tympanic membrane (ΔV_m) from its original position as a function of the pressure changes in the middle ear (ΔP_m) Neutral position=origin (α) The outward movement of the tympanic membrane is recorded in the upper right-hand quadrant and its inward movement in the lower left hand quadrant The mean of the values obtained from the temporal bones is plotted as points and their range is represented by vertical lines

operating microscope and found to be normal before the start of the experiments These were performed less than 24 hours after death During the period between the post mortem examination and the experiments the temporal bones were kept at 5°C in a humid chamber

In order to record the volume displacement of the round and oval windows a fistula was made in the superior semicircular canal The fistula was connected to an open flowmeter system of the type used by Elner et al (1971) to study middle ear mechanics This open system was used in order to avoid pressure change of the perilymph when the windows were moving

Movements of the round and oval windows caused a displacement of perilymph in the fistula which in turn led to an air flow This air flow was recorded by the flowmeter and then integrated with respect to time so that the volume displacement of round (ΔV_r) and/or oval (ΔV_o) windows could be recorded directly at pressure changes in the external ear canal (P_{ec}) or in the middle ear (P_m) The sensitivity of the flowmeter system could be so adjusted that an integrated airflow volume of 0 005 μl through the flowmeter caused a deflection of 1 mm on the ink jet recorder The calibration of the gas volume passing through the flowmeter was made before and after each experiment with the aid of an airtight micro syringe containing 1 μl Reading accuracy 1 μl±5% The linear response of the flowmeter system 0–500 μl/sec Dynamic sinusoidal response flat over 0–11 cps (Transient response 95% 20 ms) For detailed data see Elner et al (1971)

A pressure fan system (Fig 1 P_{fan}) was used to increase and decrease the pressure level in both the external ear canal and the middle ear These pressure levels were recorded by a differential pressure transducer which was calibrated with an alcohol manometer before and after each experiment Reading accuracy 1 cm H_2O±5%

RESULTS

A graph was plotted of pressure changes in cm H_2O (along the abscissa) against volume displacement in μl (along the ordinate) to demonstrate the characteristics of the tympanic membrane and the round and oval windows in the pressure range of ±20 cm H_2O After each pressure change in the ear canal or the middle ear it was checked that the tympanic membrane and window membranes returned to the neutral position, i e the origin of the graph (α) This was done to ensure that the elastic membranes were not overloaded During all the recordings the temporal bone was placed in such a position that the difference in horizontal level between the fistula and the round and oval windows was approximately 15 mm In the graphs no allowance was made for this hydrostatic pressure

1 *The volume pressure properties of the tympanic membrane*

To ensure that the elastic properties in the middle ear of the specimens used did not differ from those of normal subjects the volume dis

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VOLUME PRESSURE PROPERTIES OF ROUND AND OVAL WINDOWS

A Quantitative Study on Human Temporal Bone

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(Received September 17 1976)

Abstract A model study was carried out on human temporal bones to assess quantitatively the displacement of the round and oval windows when different pressure levels were applied to the external ear canal and inside the middle ear It was found that the major displacements of the window membranes occurred in the pressure range of ±20 cm H_2O in the intact middle ear An additional increase in the middle ear pressure up to ±70 cm H_2O caused a minor displacement of the window membranes

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MATERIAL AND METHODS

Eleven human temporal bones were taken from 5 males and 6 females aged between 57 and 90 Clinical records showed no previous otological disease The tympanic membranes and the middle ears were examined under an

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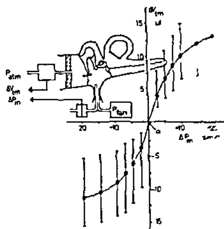


Fig 1 The displacements of the tympan ... (ΔV_{tm}) from its original position as a function ... pressure changes in the middle ear (ΔP_m) Neutr... tion=origin (α) The outward movement of the ... *membrane is recorded in the upper right-hand* ... and its inward movement in the lower left-hand ... The mean of the values obtained from the tempor... is plotted as points and their range is repre... vertical lines

kedly (compare Figs 5

... III were performed on ... Recordings IV–V were ... of the temporal bones ... in applying the poly- ... ctorily to the bony ... windows On these ... he total displacements ... was equal to the sum ... ments of the isolated ... Figs 4 and 5) This ... *of the method used*

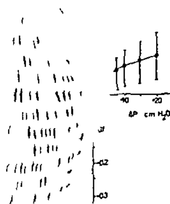
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... in open flow volume ... ng a free communica- ... ymph and the atmo- ... is technique made it ... pressure across the ... r alterations in the ... is with atmospheric ... inner ear displace

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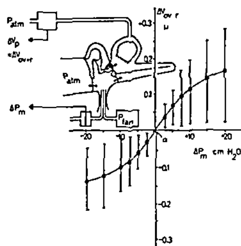


Fig 2 The volume displacement of perilymphatic fluid ($\Delta V_o + \Delta V_r$) from its original position (α) as a function of the pressure changes in the middle ear (ΔP_m) In the upper right hand quadrant the outward movements of the fluid from the inner ear are shown and in the lower left hand quadrant the inward movement The points plotted represent the mean value for eleven temporal bones and the vertical lines the range of these values

placement caused by the tympanic membrane was recorded as a function of the middle ear pressure, $\Delta V_{tm}=f(\Delta P_m)$ The results are shown in Fig 1, where the mean volumes of deviation of the tympanic membrane are plotted as points at different middle ear pressures, the range of results being shown as vertical lines

The continuous line represents the mean volume displacement of the tympanic membrane in relation to its neutral position There is no significant difference ($p>0$ 05) in the volume pressure relationship of the tympanic membrane in our temporal bone material as compared with the studies of Elner et al (1971) on normal humans

II *The perilymphatic volume displacement at changes in middle ear pressure*

The pressure was changed in the middle ear ΔP_m, via the Eustachian tube, and the displacement of the perilymphatic fluid in the fistula, ΔV_p, was recorded and was equal to the sum of the volume displacements caused by the round (ΔV_r) and oval windows (ΔV_o)

The volume–pressure properties are shown in Fig 2, where the mean volume displacements of both window membranes were recorded at different middle ear pressures It appears from this sigmoid curve that the combined volume displacement of both window membranes was 0 17 μl at +20 cm H_2O and 0 14 μ/l at −20 cm H_2O middle ear pressure

Comment In this investigation the middle ear pressure was changed up to ±70 cm H_2O which, however, produced only a slight further volume displacement of the window membranes compared with that elicited at ±2[illegible] cm H_2O

III *The perilymphatic volume displacement at changes in pressure of the external ear canal*

The pressure was changed in the external ear canal and caused the stapes to move in the oval window, which resulted in displacement of the perilymph in the fistula This volume displacement was recorded During the measuring procedure atmospheric pressure was maintained in the middle ear, whereby the round window membrane was kept in fixed position The mean relationship gave a slightly sigmoid curve with a volume displacement of 0 02 μl at +20 cm H_2O and 0 03 μl at −20 cm H_2O in the external ear canal (Fig 3

Comment In one of the investigated temporal bones the stapes caused no volume displacement when the pressure was increased in the external ear canal even though the ossicular chain was intact and mobile under microscopic control

IV *The volume–pressure properties of the round window*

For isolated recordings of volume displacements of the round window the pressure (ΔP was changed via a polyethylene tube sealed airtightly to the bony margins of the window The volume displacement was directly recorded as a perilymphatic volume displacement (ΔV_r) in the fistula The mean volume

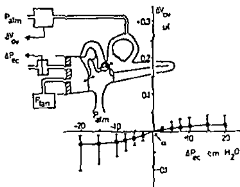


Fig 3 The volume displacement of the perilymphatic fluid (ΔV_o) from its original position (α) as a function of the pressure changes in the external ear canal (ΔP_{ec}) In the upper right hand quadrant the outward movement of the fluid from the inner ear and in the lower left hand quadrant the inward movements Atmospheric pressure is maintained in the middle ear The points represent the mean value from eleven specimens and the vertical lines the range of results

pressure properties of the round window are represented in Fig 4 It appears from the figure that the mean volume displacement inwards reached 0 12 μl at +20 cm H_2O and 0 12 μl outwards at −20 cm H_2O pressure applied to the outside of the round window

V *The volume–pressure properties of the oval window*

The isolated volume–pressure characteristic of the oval window was studied in two ways In the first recording the ossicular chain was intact The tympanic membrane was perforated and the round window connected to the atmosphere via a polyethylene tube (see Fig 5) When the pressure in the middle ear was changed via the external ear canal the oval window alone was affected The volume displacement of the oval window was recorded as a perilymph volume displacement (ΔV_o) Fig 5 illustrates the mean volume–pressure characteristics of the oval window with an intact ossicular chain The junction between the stapes and incus was broken and the stapedius tendon cut (see Fig 6) Thus there was no restrictions of movements by the ossicular chain, ligaments or middle ear muscles In this way the volume displacement in the oval window increased markedly (compare Figs 5 and 6)

Recordings I, II and III were performed on all temporal bones Recordings IV–V were performed on only five of the temporal bones due to the difficulties in applying the polyethylene tube satisfactorily to the bony margins of the round windows On these five temporal bones the total displacements of perilymph (Fig 2) was equal to the sum of the volume displacements of the isolated window membranes (Figs 4 and 5) This illustrates the accuracy of the method used

DISCUSSION

In the present study an open flow volume method was used involving a free communication between the perilymph and the atmosphere via the fistula This technique made it possible to quantify the pressure across the window membranes after alterations in the middle ear presence Thus with atmospheric pressure maintained in the inner ear, displace

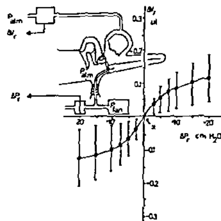


Fig 4 The volume–pressure characteristics of the round window $\Delta V_r = f(\Delta P_r)$ recorded via the perilymphatic fistula The origin of the graph represents the window s normal position (α) In the upper right hand quadrant the inward membrane movements e g movements towards the inner ear are shown In the lower left hand quadrant the outward membrane movements e g towards the middle ear are shown The mean values of the results obtained from five temporal bones are plotted as points and their range is shown as vertical lines

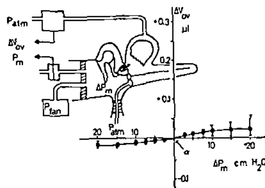


Fig 5 The volume–pressure characteristics of the oval window with intact ossicular chain $\Delta V_o = f(\Delta P_m)$ recorded via the perilymphatic fistula The origin (α) represents the neutral position of the window The mean values from five temporal bones are plotted as points and the range is shown as vertical lines

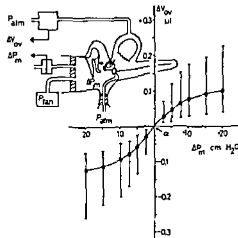


Fig 6 The volume–pressure characteristics of the window, $\Delta V_o = f(\Delta P_m)$ with the tendon of the stape muscle cut and the junction between the stapedius incus broken The origin of the graph represents window s neutral position (α) The points plottec present the mean value from five temporal bones anc vertical lines the range

ment of volumes of perilymph were recorded which we equate with the volume displacement caused by the window membranes

The total round and oval window displacement was about five times greater at pressure changes in the middle ear than at equivalent pressure changes in the external ear canal (compare Figs 2 and 3) Most volume displacement took place in the range of ±20 cm An increase in the pressure to ±70 cm produced only a further slight displacement of the membranes so long as the inner ear was exposed to atmospheric pressure When the tympanic membrane system was intact a pressure change in the middle ear produced a very limited displacement of the oval window, approximately one sixth of that of the round window (Figs 4 and 5) When the junction between the stapes and incus was broken, the oval window displacement capacity became almost equivalent to that of the round window (Figs 5 and 6) Thus, on the whole, these results confirmed those obtained by Politzer (1861) and Bezold (1908)

Only when the inner ear is open to atmospheric pressure these studies provide quantitative information about the displacement of the round and oval windows If the inner ear is regarded as a closed rigid chamber and the window membranes the only elastic elements, a pressure change in the middle will be directly and quantitatively transfe to the inner ear via the windows The in ear, however, has several volume compen ing factors, the cochlear aqueduct, the ves ular aqueduct and the blood vessels, wh makes pressure transmission to the inner rather more complex

The present investigation forms part o series of studies in progress in the departm on the pressure transmission between middle and the inner ears (Ingelstedt et a 1974, Densert et al , 1976, Ingelstedt et a 1976, Casselbrant et al , 1976)

ZUSAMMENFASSUNG

In einer Modellstudie an menschlichen Schlafenbein paraten wurden im außeren Gehörgang und innerhalb Pauke eine Reihe von verschiedenen Druckpegeln ap ziert und die hierdurch hervorgerufenen Lageveran rungen des runden und ovalen Fensters quantitativ erfa Es ergab sich daß die großten Lageveranderungen Fenstermembranen bei Druckpegeln im Umfang von ± cm H_2O im intakten Mittelohr auftraten Ein zusätzlic Anstieg des Mittelohrdrucks bis auf ±70 cm H_2O rief eine geringe Lageveranderung in den Fenstermembra hervor

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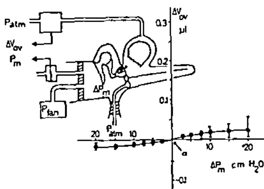


Fig 5 The volume–pressure characteristics of the oval window with intact ossicular chain $\Delta V_o = f(\Delta P_m)$ recorded via the perilymphatic fistula The origin (α) represents the neutral position of the window The mean values from five temporal bones are plotted as points and the range is shown as vertical lines

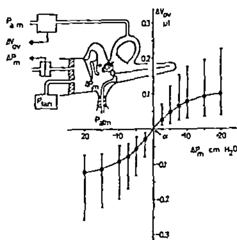


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One of the major reasons for these discrepancies is probably the difference in "sensitivity" of the different 'measurements". For example, electron microscopy will reveal more subtle pathological changes than either light microscopy or behavioral audiometry Further, there has been variation in the species of animals used the frequency, duration, and intensity of sound stimuli, and number of ears exposed (unilateral vs bilateral) From the morphological point of view, these investigations have mainly been concerned with hair cell loss and possible loss of neural elements visible in light microscopy The mechanism of this destruction is, as yet, not well understood Doubtless, physical force exerted by intense vibratory energy is sufficient to cause tearing and dislocation of cochlear tissues Spoendlin (1971) suggested that these "changes can only partly be explained on the basis of a purely mechanical effect" The organ of Corti may also be damaged secondarily to influences on the cochlear vessels i e, an impaired oxygen supply during increased need (Perlman & Kimura, 1962, Lawrence et al, 1967, Hawkins, 1971, Hawkins et al, 1972, Johnsson & Hawkins, 1972, Lipscomb & Roettger, 1973, Henderson et al 1974)

The present investigation studied the influence of noise on the cochlea of the chinchilla as measured by structural changes in the cochlear neuroepithelium as well as by functional changes reflected audiometrically These data will be projected to possible changes in cochlear vasculature

MATERIALS AND METHODS

Behavioral testing

Pure tone auditory thresholds of 10 young chinchilla were obtained using an avoidance conditioning method This method first described by Miller (1970) was modified to reduce training and testing time Each animal underwent testing while located in a custom-built double-walled sound treated chamber (Roettger et al, 1976) Appropriate signal level calibration procedures were employed to assure accuracy of signal presentations within the test booth

Avoidance training was initiated whereby the animal was taught to avoid a shock by jumping the barrier at the onset of a conditioning tone (2 000 Hz at 60 dB SPL)

Audiograms were obtained after suitable training responses were achieved Each pre-exposure pure tone threshold was recorded as the average of measures taken on 3 successive days Baseline audiograms for the frequencies 500, 1 000, 1 500, 2 000, 3 000, 4 000, 6 000 and 8 000 Hz were used to qualify the animal for inclusion in the study, and were required to be within ±10 dB of the norms for the chinchilla reported by Miller (1970)

Noise exposure

Once baseline audiograms were completed, each animal was placed in another sound treated custom built double-walled chamber to be exposed to broad-band noise (110 dB SPL) for an eight hour period The spectrum of sound in the chamber was found to be relatively flat from 63 to 4 000 Hz as can be seen in Fig 1

Immediately after exposure to high level sound, each animal was re-tested to note the maximum threshold shift The animals were re-tested daily until the hearing had stabilized (8 to 16 days post-stimulation) The remaining loss was considered to be the permanent threshold shift (PTS) caused by the noise exposure

Control animals

Ten young binaural chinchilla served as controls for studying cochlear vasculature and sensorineuroepithelium The histological preparations used to study representative parts of the cochlear sensorineuroepi'
in the control animals was simi
used in the noise-exposed cl

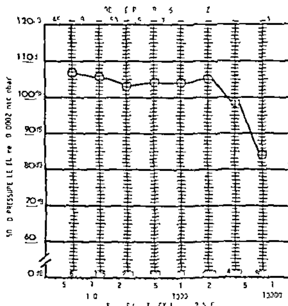


Fig 1 Octave band analysis of the noise stimulus illustrating that the spectrum was quite flat especially in the mid frequency range

portion of the study was reported recently (Axelsson & Lipscomb, 1975)

Histological study

Upon completion of all post-stimulation hearing tests (8–16 days) the chinchilla cochlea was prepared for histological study according to methods described previously (Axelsson et al, 1974, 1975, Axelsson & Lipscomb, 1975) The dissection procedure began with a mid- or para modiolar section of the cochlea The cut surface was thoroughly examined for displacement of the vestibular membrane occurrence of hemorrhage, etc After further dissection, vessels as well as other structures of the external wall were examined for evidence of pathological changes In addition, specimens of the spiral lamina were viewed from the basal side for close examination of the spiral lamina vessels and then from the apical side for the study of the organ of Corti Missing hair cells and hair cells replaced by the collapsed phalangeal processes of the Deiter cells were noted as "damaged" on the cytocochleogram (Engstrom et al, 1966) Contrary to some previous investigations, disfiguration of stereocilia, displacement of cell nuclei, and similar changes were not considered as damaged cells Representative findings were documented with phase contrast photomicrography

RESULTS

Behavioral audiometric findings

Figs 2–4 show averaged audiometric results obtained on the ten animals studied Fig 2 shows average pre-exposure thresholds (in dB SPL) and the ranges (brackets) for the group It was noted that the average range for the baseline (pre exposure) audiograms was 11 3 dB for all test frequencies with no frequency having more than a 15 dB range This indicated good correspondence for all animals with regard to their initial hearing sensitivity

Average maximum threshold shifts immediately after noise exposure are seen in Fig 3 These data represent a single retest for each animal before recovery took place The noise exposure resulted in sizeable hearing threshold shifts for all animals with a somewhat greater range among animals than was seen in the pre exposure tests The range

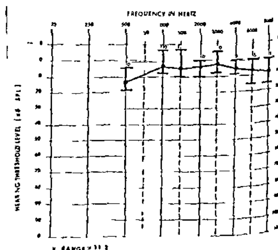


Fig 2 Average pure tone thresholds and ranges for the ten experimental animals prior to sound stimulation (baseline audiograms)

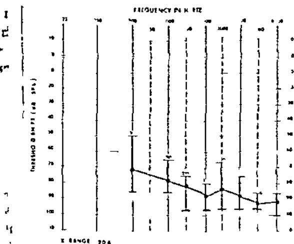


Fig 3 Average pure tone threshold shifts and ranges for all animals immediately after exposure to the high intensity sound

was as great as 35 dB (500 Hz) averaging 20 6 dB for all test frequencies

The PTS measured after temporary threshold shift (TTS) recovery was found to have the greatest average range regardless of test frequency. The average range was 34 4 dB for all frequencies Ranges for each frequency are shown in Fig 4 Although the animals entered the experiment with highly similar hearing and demonstrated somewhat comparable maximum threshold shifts immediately post-exposure, post-stimulation recovery was considerably greater for some animals than for others

Histopathological results

General findings A great deal of individual variability was noted in the histological appearance of the preparations even though the same technique was used for injection, fixation, decalcification and counterstaining

Of the 10 animals, 2 injected excellently, 2 others injected satisfactorily, and the remaining 6 were only partially injected by the contrast medium In general, the contrast injection was considerably less favorable in these chinchilla than in non noise-exposed counterparts

Minute black droplets were found in some cochleas in the scala vestibuli and scala tympani, probably indicating the presence of lipid osmiophilic substances The position of the vestibular membrane was, in most cases similar in the two ears In 7 ears, the vestibular membrane was distended (bulging into scala vestibuli) throughout the cochlea In 3 ears the vestibular membrane was collapsed In 6 ears, the vestibular membrane was distended in some parts of the cochlea and was collapsed in other parts The appearance of the vestibular membrane in the remaining 4 ears was essentially normal It cannot be determined whether this finding is an artifact caused during the initial bisection of the cochlea or the result of noise stimulation creating a condition wherein the vestibular membrane is forced out of its normal position

Sensorineuroepithelium

The organ of Corti showed, in general, hair cell damage somewhat greater apically than basally, and more frequent in the third row of outer hair cells, decreasing toward the inner hair cells (Fig 5) In some cases, pathology was concentrated in localized areas, whereas other areas in the same half turn were completely normal Stereocilia could not always be seen Whether this was

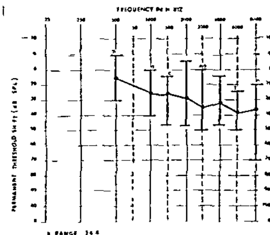


Fig 4 Average permanent threshold shifts and ranges after the hearing had recovered to a stabilized level

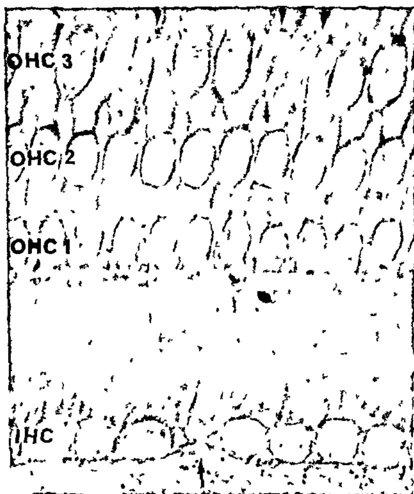


Fig 5 Chinchilla spiral lamina 3rd turn In the noise exposed animal phalangeal scars are most frequently seen in the third row of outer hair cells (*OHC*) shown by arrows Missing inner hair cells (*IHC*) are less common (*arrow*)

ue to differences in the technical procedure when removing the tectorial membrane or other factors remains unsolved However in those tissues where stereocilia were seen, they had, in general, a normal configuration In areas adjacent to concentrated hair cell damage, the stereocilia appeared to have an altered configuration They were seen to have a more clotted or clumped appearance they were tilted to either side of the hair cells, or they had a generally uneven appearance These findings were most common in the third row of outer hair cells and less prevalent in the first and second rows

Inner hair cells in general, showed much less change than did the outer hair cells A fairly common finding was an apparent displacement of the nucleus toward the upper surface of the cell (Fig 6) In a few cases, some hair cells were missing however, this was never seen to occur over a large area Irregular stereocilia of the inner hair cells were found in 7 cochleas There were very seldom any abnormalities in the supporting structures

Vasculature The vessels of the spiral lamina always appeared normal Histopathological findings in the external wall were found in the stria vascularis Often the surface cells of the stria vascularis appeared uneven swollen or shrunken with intercellular gaps a finding which was particularly frequent apically, decreasing toward the base of the cochlea Another common finding (in 10 cochleas) was what appeared to be vacuoles in the stria vascularis This condition was present in all turns in localized areas but was most common in the third turn

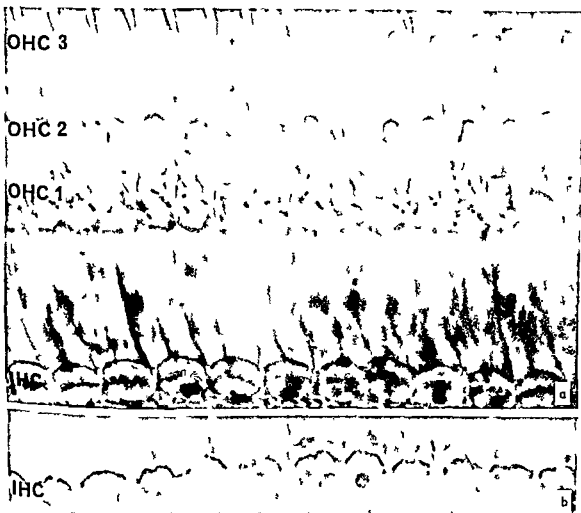


Fig 6 Ch nchilla sp ral lamina 2nd turn In the no se exposed an mal d placement of the nucle of the nner hair cells (*IHC*) towards the cuticular surface can be seen along with intact stereoc lia (*a* and *b*) In the first row of outer hair cells (*OHC*) the stereoc lia appear d sfigured (*a*)

and in the apical parts of the stria vascularis (Fig 7)

Ha r cell counts

The results of ha r cell counts are shown in Table I Statistical analysis of the cell counts indicated sign ficant differences (0 01 levels of confidence) in the amount of cell damage in the apical coil when compared with both the m ddle and basal turns of the cochlea Differences between the amount of hair cell damage in the m ddle and basal coils were non s gnificant S m larly cell count differences between ears of the same animal were not statistically s gnificant For the apical coil the percentage of cell damage ranged between 14 1% and 2 6% with a mean of 6 3% For the middle coil the range was between 9 5% and 0 9% with a mean of 3 3% Cell damage in the basal coil ranged between 12 9% and 0 6% with a mean of 3 2%

Aud ometric and histologic comparison

In Fig 8 behavioral hearing test results (PTS) and h stological data are shown for

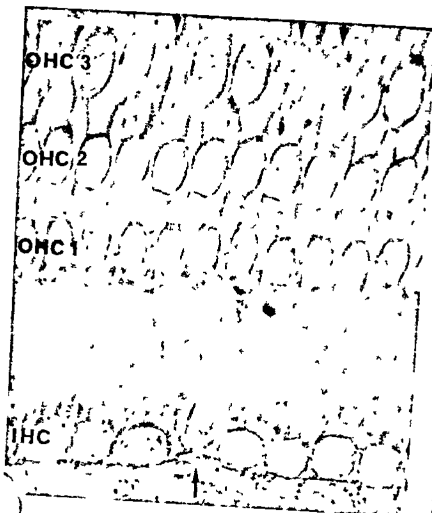


Fig 5 Chinchilla spiral lamina, 3 turn In the noise exposed animal, phalangeal scars are most frequently seen in the third row of outer hair cells (*OHC*) shown by arrows Missing inner hair cells (*IHC*) are less common (arrow)

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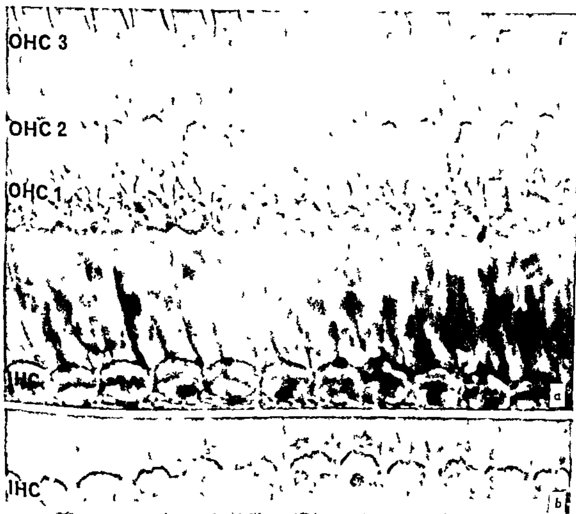


Fig 6 Chinchilla spiral lamina 2nd turn In the noise exposed animal displacement of the nuclei of the inner hair cells (*IHC*) towards the cuticular surface can be seen along with intact stereocilia (*a* and *b*) In the first row of outer hair cells (*OHC*) the stereocilia appear disfigured (*a*)

and in the apical parts of the stria vascularis (Fig 7)

Hair cell counts

The results of hair cell counts are shown in Table I Statistical analysis of the cell counts indicated significant differences (0 01 levels of confidence) in the amount of cell damage in the apical coil when compared with both the middle and basal turns of the cochlea Differences between the amount of hair cell damage in the middle and basal coils were non significant Similarly cell count differences between ears of the same animal were not statistically significant For the apical coil the percentage of cell damage ranged between 14 1% and 2 6% with a mean of 6 3% For the middle coil, the range was between 9 5% and 0 9% with a mean of 3 3% Cell damage in the basal coil ranged between 12 9% and 0 6% with a mean of 3 2%

Audiometric and histologic comparison

In Fig 8 behavioral hearing test re (PTS) and histological data are sh



Fig 7 Chinchilla cochlea external wall 2nd turn In noise exposed animals large vacuoles sometimes appear immediately above and below the attachment of the vestibular membrane (*arrows*) (*SVS*) Stria vascularis

able 1 *Results of cochlear hair cell count for all animals*

Animal	3rd turn *D*	3rd turn *I*	2nd turn *D*	2nd turn *I*	Basal turn *D*	Basal turn *I*	Total cells
RC 12 R	5 18	94 82	1 16	98 84	1 60	98 40	6 988
RC 12 L	4 61	95 39	1 74	98 26	6 82	93 18	8 301
RC 13 R	3 02	96 98	1 39	98 61	87	99 13	7 317
RC 13 L	3 79	96 21	92	99 08	2 19	97 81	6 799
RC 15 R	6 64	93 36	4 71	95 29	1 20	98 80	8 921
RC 15 L	9 17	90 83	5 56	94 44	2 83	97 17	7 383
RC 16 R	11 22	88 78	4 60	95 40	2 85	97 15	6 177
RC 16 L	11 62	88 38	1 83	98 17	6 73	93 27	7 847
RC 18 R	14 15	85 85	2 62	97 38	80	99 20	9 067
RC 18 L	6 09	93 91	8 31	91 69	1 79	98 21	7 997
RC 20 R	2 61	97 39	1 54	98 46	2 03	97 97	7 047
RC 20 L	4 07	95 93	1 17	98 83	1 60	98 40	8 088
RC 21 R	2 64	97.36	2 63	97 37	2 50	97 50	5 313
RC 21 L	3 72	96 28	3 00	97 00	11 43	88 57	6 245
RC 22 R	7 33	92 67	1 92	98 08	3 15	96 85	7 260
RC 22 L	3 41	96 59	1 34	98 66	64	99 36	8 810
RC 23 R	4 14	95 86	9 01	90 99	91	99 09	7 493
RC 23 L	5 95	94 05	9 49	90 51	12 91	87 09	6 355
RC 24 R	6 68	93 32	1.80	98 20	99	99 01	8 234
RC 24 L	9 65	90 35	2 12	97 88	57	99 43	6 170

each of the 20 noise exposed ears used in the study The amount of PTS at each frequency is plotted in dB on the left of each chart while intact hair cells are plotted in percent on the right margin Placement of the cell count figures was arbitrary since absolute comparisons between loss for tones and loss of sensory cells cannot be made Of importance in the comparison are the relative configurations of PTS and cell count data

Many of the inconsistencies of previous investigations were confirmed by the present study Hearing in the low frequencies remains essentially unaffected by discrete apical hair cell loss Hearing in this range was essentially within normal limits (7 of 10 animals) and did not exceed 30 dB These findings correspond to a discrete hair cell loss both in the normal hearing individuals (maximum 11%/12% cell loss in the right and left ear respectively) and in those with discrete hearing loss (maximum 14%/6% cell loss) Concerning the middle turn (mid frequencies) 2 animals had normal hearing and 8 had a moderate hearing loss between 28–46 dB The 2 chinchilla with normal hearing post-exposure also had normal cell counts (1%/2% and 5%/6% loss respectively) In the animals with hearing loss the cell counts in general were also normal the greatest loss being 9%/9% in an animal with approximately 45 dB loss in the mid frequencies

The basal turn (high frequencies) was markedly different from the apical turn in that no animal showed normal hearing while at the same time cell counts invariably showed normal findings with a maximum loss of 1%/13% in one animal or 3%/7% in another

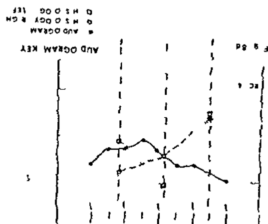
DISCUSSION

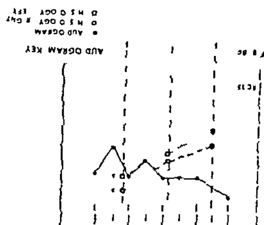
The present investigation adopted a modification of a widely used technique (Miller 1970) for obtaining behavioral audiograms in the chinchilla The advantage of this modification is the shortening of the time required to achieve a reliable audiogram without losing any consistency in response Details on this modification will be presented elsewhere (Roettger et al 1976)

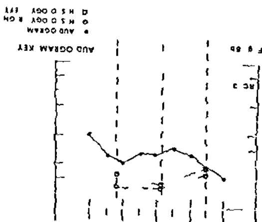
Binaural chinchilla were used in this study in order to maximize the number of ears and to minimize the time needed to obtain behavioral threshold data Miller (1970) reported that the average sensitivity of binaural chinchilla ranges between 2 to 8 dB better than that of the monaural animal These sensitivity differences were deemed to be relatively insignificant in light of the fact that each animal served as his own control If there had been striking variation between ears for each animal in terms of hair cell loss the observations from this study would be in question However only slight differences between ears was demonstrated Thus it is felt that the results of this study are valid especially as they relate the audiometric to histologic data Broadband noise of 110 dB SPL was used since from our experience this results in a clearly detectable audiometric loss (PTS) as well as in definite histological changes in the hair cells

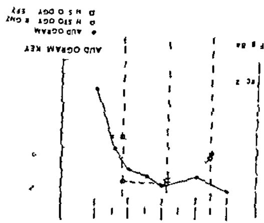
Daily audiograms were obtained after noise exposure as long as 16 days post exposure until three consistent audiograms had been obtained on each animal The animals were terminated soon after the last test

Histological observations were quite clear cut and showed little variation between ears for the 10 animals used Generally sensory cell damage was greatest in the apex and decreased toward the base of the cochleas an observation in agreement with earlier reports (Lipscomb 1972 Lim & Melnick 1973) Lesions were more frequent in the outermost row of outer hair cells and were fewer toward the inner hair cells also in agreement with other published data (Engstrom et al 1966 Hunter Duvar & Elliot 1972 1973) Swollen outer hair cells were noted A similar finding was reported by Ward & Duvall (1971) Vacuoles and inter









ellular spaces between the surface cells of the stria vascularis similar to those described by Ward & Duvall (1971) were also identified in this study The reduced vascular lumena in the external cochlear wall observed by Ward & Duvall (1971) were not apparent in our specimens The finding that the nucleus of the inner hair cells is displaced towards the upper surface of the cell has also been found previously (Hunter Duvar & Elliot 1972)

The vestibular membrane was noted to be distended as well as collapsed in many chin chilla Abnormal placement of the vestibular membrane was noted in all but 4 ears in this study The possibility must be considered that the displacement could have been due to one or more of the preparatory steps e g the injection of fixative the bisection of the cochlea However, care was always taken to infuse the fixative slowly in the apex and oval window We feel that this would not result in any changes in the position of the vestibular membrane particularly not a distention The bisection of the decalcified cochlea with the razor blade could have displaced the vestibular membrane at or near the locus of the cut Previous authors have seldom commented on the position of the vestibular membrane with the exception of noting that it ruptured after high intensity sound stimulation (Eldredge et al 1957 Lawrence & Yantis 1957) Consequently the influence of noise exposure on the position of the vestibular membrane remains to be elucidated

Audiometric results showed large interindividual differences This confirms the almost

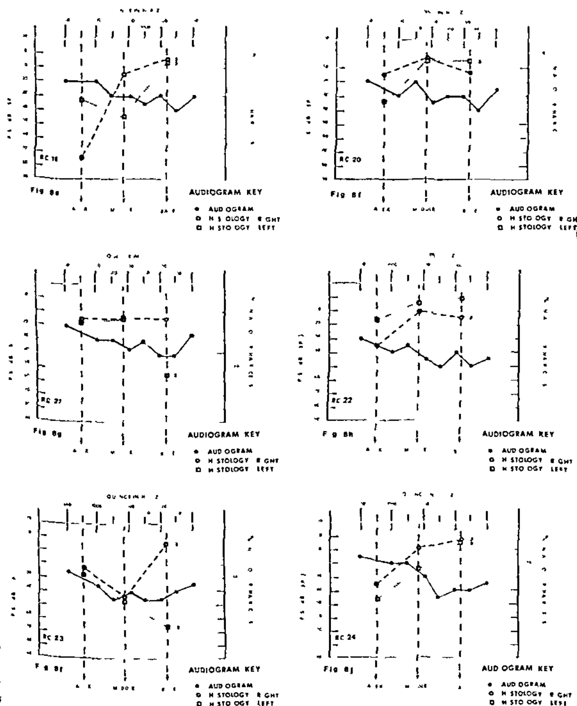


Fig 8a-j Composite illustration combining PTS data and hair cell counts for 10 noise exposed chinchilla Binaural PTS (●—●) relates to scales at the top and left margins Percentages of intact hair cells (right ○- ○ left □ □) are displayed for each turn of the cochlea and relate to the scales on the right and lower margin The relative position of right and left scales is arbitrary

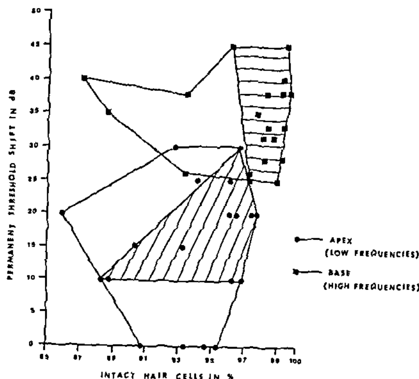


Fig 9 Permanent threshold shifts and intact hair cells for each animal for the basal turn (4 000–8 000 Hz) and the apical turn (500 Hz) The majority of data points fall within two distinct shaded areas Hearing is better in the low than in the high frequencies in spite of greater hair cell loss in the apical than in the basal turn

uniform experience of a large individual variation in sensitivity to noise A summary of the audiometric and histologic agreement for both apical turn low tones and basal turn high tones is shown in Fig 9 Although there is some dispersion, the majority of points for these cochlear regions fall into two distinct areas (shaded by parallel lines) The present investigation demonstrated the consistent agreement between audiological and histological (haircell counts) findings concerning low tones apical turn where minimal hair cell loss was accompanied by essentially normal hearing Contrarily, in the basal turn, histology was essentially normal, whereas all animals showed some loss for high tones

One reason for these discrepancies may be some shortcoming in the observation technique used in light microscopy We opted to err in the direction of a conservative cell counting protocol and counted as damaged only those hair cells obviously scarred or missing Therefore, cells with displaced or fused cilia or nuclear alterations or swollen cells may or may not have been functional in the auditory system This explanation however, would apply to hair cells located throughout the cochlea, thus the better correlation between audiograms and histology apically than basally remains We believe that one explanation for these differences may be related to vascular conditions

It is known that the base of the cochlea differs from the apex The basal turn in mammals appears to have a much more elaborate vascular supply than the apical turn with its seemingly pronounced simplification of the vasculature This then would indicate a better ability for oxygen to be supplied basally than apically However, the vessel length per tissue area of the stria vascularis is similar basally and apically (Axelsson 1968) Further, the organ of Corti relies primarily on oxidative metabolism However the metabolic rate is greatest in the basal turn (Koide et al , 1964, Meyer zum Gottesberge et al , 1963, Mizukoshi & Daly, 1967) Glycogen, the most important single contribution to the total energy reserve of the organ of Corti, is distributed in inverse relationship to the glucose pool, being higher apically than basally (Spector & Lucen-

1974) Further, P-creatinine, another energy source, decreases more rapidly in the basal turn than apically during ischemia Also, according to Falbe Hansen & Thomsen (1963) the ability to carry out anaerobic metabolism is less well developed basally than apically All these data tend to explain the greater vulnerability of the basal cochlear turn to all kinds of trauma resulting in a metabolic deficiency with irreversible tissue damage This tissue damage need not manifest itself in damage to hair cells, as can be seen in Fig 8

In general, we found that the vascular system of the cochlea did not accept injection with contrast particularly well Age, mechanical lesions and barotrauma have previously been found to impair contrast medium injection (Axelsson, 1971, Axelsson & Hallen, 1973, Lamkin et al , 1975) To the list we suggest the addition of noise exposure We did not, however, find any major vascular differences between the basal and apical turns using light microscopy and moderate magnification In addition to the above-cited indirect evidence of a vascular contribution to high frequency hearing loss, more substantial evidence of possible vascular anomalies should be examined with the aid of high power phase-contrast and electron microscopy Such investigations are proceeding now in our laboratories

ZUSAMMENFASSUNG

Das Gehör zehn junger Chinchillas wurde geprüft Die Tiere wurden dann 8 Stunden lang mit 110 dB (SPL) Breitbandgeräusch beschallt Das Gehor wurde täglich nach Abschluß der Exponierung geprüft bis eine permanente Gehörschwelle erreicht wurde Die Cochlea wurde danach mit Häutchenpräpariermethode studiert und Haarzellen und Gefäße untersucht Die Ergebnisse waren eine unregelmäßige Stellung der Reißner schen Membran unvollständige Kontrastfüllung der Gefäße mäßige Degeneration der außeren Haarzellen und Verlagerung der Kerne der inneren Haarzellen Leichtere Zellschaden in der basalen Windung liefen parallel mit größeren Gehörverlusten im hohen Frequenzbereich als man von der Histologie her erwarten konnte

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COMPARATIVE SURFACE STUDIES OF OTOTOXIC EFFECTS OF VARIOUS AMINOGLYCOSIDE ANTIBIOTICS ON THE ORGAN OF CORTI IN THE GUINEA PIG

A Scanning Electron Microscopic Study

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(Received August 13 1976)

Abstract It was the purpose of this study to establish criteria for use in comparing the toxic effects of aminoglycosid antibiotics on the organ of Corti by means of scanning electron microscopy Amikacin Tobramycin and Gentamicin were administered twice a day subcutaneously for 10 days to healthy guinea pigs One group of animals was sacrificed 1 day after completion of the treatment the other group was allowed to survive 22 days Depending upon the dosage of the administered drug Amikacin (150 mg per kg body weight daily corresponding to 10 times an average recommended human dose) caused pronounced outer hair cell damage even 1 day after the treatment was stopped At this time Gentamicin and Tobramycin (150 mg per kg body weight daily corresponding to 50 times an average human dose) showed less damage After 22 days survival late toxic effects were found mainly in Gentamicin and Tobramycin treated animals After 3 weeks nearly total outer hair

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cells 300 mg per kg body weight Amikacin (i e 20 times the average human dose) showed about the same toxic effect on sensory cells of the guinea pig as did 150 mg Gentamicin or Tobramycin per kg body weight We are conscious of the fact that there are problems in correlating the weight of a drug and its probable toxic effect In comparative animal experiments we consider it useful to standardize the time of exposure the amount of drug administered (e g related to the human dose) and the survival time

Hinshaw & Feldman (1945) published an early report on the toxic effects of streptomycin in the treatment of tuberculosis Floberg et al (1949) found toxic effects of streptomycin on the sensory epithelium of the inner ear of the guinea pig Wersall & Hawkins (1962) were the first to study the toxic effects of streptomycin on the feline labyrinth by means of electron microscopy Hawkins & Engström (1964), Kohonen (1965) and Kellerhals et al (1967) studied ototoxic effects by means of surface preparations in combination with transmission electron microscopy Wersall et al (1973) reported ototoxic effects of Gentamicin in the guinea pig as revealed by the combination of interference contrast, light microscopy, scanning and transmission electron microscopy Recently Bagger-Sjoback & Wersall (1976) reported toxic effects of Gentamicin on the basilar papilla of the lizard After 14 days of treatment with 100 or 150 mg per kg body weight, severe damage to the sensory cells was studied with scanning EM By means of TEM we studied the toxic effects of aminoglycoside antibiotics on the cochlear nucleus of the guinea pig (Theopold, 1976) Nearly all of the above mentioned authors performed the animal experiments under different conditions In morphological studies on the effects of antibiotics in the inner ear, the daily dose varied from 3 mg per kg body weight Gentamicin administered in two subcutaneous doses up to a single dose of 1 000 mg per kg body weight Kanamycin There are reports on long

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term exposure with sublethal dosage and sacrifice immediately after the experiment, varying up to 10 months in their survival times, as reported by Ylikoski (1974) After treatment with very high doses of aminoglycoside antibiotics it must be discussed whether the effects which are reported regarding the damage to the sensory epithelium are primary toxic effects or are caused secondarily by a general intoxication due to the kidney damage in the experimental animal

In our studies on the neurotoxic effects of aminoglycoside antibiotics in the cochlear nucleus (Theopold, 1976) we tried to standardize our animal experiments in order to be able to compare the varying potency of ototoxic antibiotics with the morphological results which we expected to find in the cochlear nucleus as well as in the organ of Corti of the guinea pig

MATERIAL AND METHODS

Twenty-one healthy young guinea pigs of about 200 g (6 additional animals as a control) ere given daily s c injections of Gentamicin, bramycin and Amikacin Gentamicin and obramycin were given mainly in the high *dosage of 150 mg per kg body weight*, which corresponds to a 50-fold higher administration than the recommended average human dose Amikacin was injected with 150, 300 mg per kg body weight, corresponding to a dosage 10–20 times higher than the recommended human dosage All animals were treated for 10 days by two s c injections The animals were, in principle, allowed to survive either 1 or 22 days One animal treated with 150 mg per kg body weight Amikacin survived 5 days, another one which is included in this study survived 28 days As antibiotics we used Gentamicin (Refobacin® Merck, Sulmycin® Byk-Essex), Tobramycin (Gernebicin®, Lilly), Amikacin (Biklin® Bristol Grunenthal) All animals were sacrificed under Nembutal anesthesia by intracardial fixation with 2 5% glutaraldehyde (for further details see (Theopold, 1975) Post fixation of the ears was performed in buffered 1% OSO_4 The right ears were processed for SEM while the left ears were used for other methods such as surface preparation or TEM Further processing of the specimen was done by alcohol dehydration critical point drying, and sputtering with a thin gold layer The specimens were examined in a Cambridge Stereoscan Mark 2 A

Plate 1 Fig 1 Normal appearance of outer hair cells in the 3rd coil from basal (Cf Fig 7) Gentamicin treatment 150 mg/kg body weight for 10 days 22 days survival time ×7 120 *Fig 2* Survey micrograph of 3rd coil from base Arrows indicate damage probably in the row III of outer hair cells Supporting elements moved into the vacant space (Cf Fig 10) Tobramycin treatment 150 mg/kg body weight 1 day survival ×230 *Fig 3* Basal coil of a Tobramycin treated animal 150 mg per kg body weight Here we found hardly any damage just one collapsed outer hair cell in row III Inner hair cells look well preserved (*IHC*) ×2 750 *Fig 4* The supporting elements the Deiter s cell (*DC*) and their phalangeal process (*Ph*) show no OHC (outer hair cells) IHC (inner hair cells) damage The specimen is from the same animal as in Figs 2 and 3 ×2 625

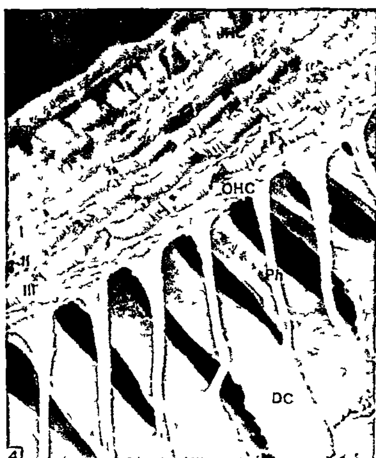
Plate 2 Fig 5 Grape like form of degenerated hairs in row II of the outer hair cells This animal GP 154 showed comparatively little ototoxic effect Gentamicin treatment 150 mg per kg body weight for 10 days 22 days survival 1st coil from basal ×5 400 *Fig 6* Cytoplasmic protrusions (*CP*) in damaged outer hair cells with fused hairs (×) One giant form of a hair is found *Compared with the basal coil the damage is more* widespread 3½ coils from basal GP 154 same animal in Fig 5 ×5 000 *Fig 7* Widespread severe damage in the outer hair cells (*OHC*) The inner hair cells (*IHC*) show no obvious change Gentamicin treatment 150 mg per kg body weight for 10 days *22* days survival *×623 Fig 8* Clumping of single hairs (×) incipient "grapelike" formations (Cf Fig 5) Cytoplasmic protrusion (*CP*). Same animal as in Fig 7 ×6 875

Plate 3 Fig 9 Only slight damage in the basal coil of an Amikacin treated animal Some forms of degenerated hairs (*arrows*) B 305 Amikacin treated 300 mg per kg body weight for 10 days 22 days survival ×1 050 *Fig 10* Severe damage in the basal coil with loss of outer hair cells and the supporting structures (within the ar rows) Inner hair cells (*IHC*) pillar cells (*PC*) Hensen cells are visibly not affected GPB304 same exposure and survival time as in the animal in Fig 9 Magnification ×1 800 *Fig 11* Hair fusion (×) beginning at the tops of the hairs Cytoplasmic protrusion (*CP*) in an outer hair cell with distated hairs GPBB3 150 mg per kg body weight daily for 10 days 5-days survival ×6 875 *Fig 12* Hair fusion (×) as an early sign of degeneration From two outer hair cells the hairs are missing (*arrow*) GPBB3 same animal as in Fig 11


1


2


3


4
OHC
Ph
DC



5


X
X
CP
6


7


X
CP
8


9




CP
X
11


X
12


CP
13


14


5


16

RESULTS

After the 10-day treatment with 150 mg per kg body weight Gentamicin, Tobramycin, or Amikacin, the organ of Corti showed little change when the animals were sacrificed after 1 day At this time, the toxic effects were rarely found in the basal coil (Figs 2 3), whereas the 3rd coil of the same animal showed definite hair cell loss in rows II and III of the outer hair cells Where hair cells in row II disappeared, their places were taken by cells which moved over from row III (Fig 2)

In Amikacin treated animals sacrificed after short survival times and low dosage, sensory hair damage was observed only sporadically In one animal we found hair fusion even after the administration of 15 mg per kg body weight Amikacin (Fig 14) After treatment with 150 mg Amikacin for 10 days we observed hair fusion which seemed to begin at the top of the hairs (Fig 12), in other hair cells we observed cytoplasmic protrusions (Fig 11)

Longer survival times (22 days)

After longer survival times (22 days) we now found more pronounced damage to the outer hair cells, occurring generally in the basal coil (Figs 7, 9, 10) There was a near total loss of the outer hairs, whereas the inner hair cells seem to be well preserved (Figs 7, 10) Supporting cells moved into the empty spaces of lost outer hair cells The degree of difference in cell damage was quite remarkable, even after identical treatment and survival times (For Gentamicin see Figs 5, 7, for Amikacin see Figs 9, 10) Inner hair cell loss was never found, except in some apical parts

Gentamicin/Tobramycin treated animals showed a characteristic pattern of hair damage after 22 days Various forms of moderately affected hairs (Fig 8) In one and the same animal we found all forms of degenerating hairs (Figs 7, 8), and even preserved areas (Fig 1) A grape like form (Fig 5) resulted from the degeneration of single hairs (Fig 8) Other outer hair cells showed giant forms of stereocilia (Figs 6, 13)

Amikacin induced damage to outer hair cells was often found as a swelling or ballooning of the hairs (Figs 14, 16) Cytoplasmatic protrusions were found early in Amikacin-treated animals, but also terminally after Gentamicin/Tobramycin treatment (Fig 6)

Plate 4 Fig 13 Formation of giant hairs in a damaged outer hair cell of row II Cytoplasmic protrusion (*CP*) is discernible All the other cells are only slightly affected Gentamicin-treated animal 150 mg per kg body weight for 10 days 22 days survival ×5000 *Fig 14* Ballooning in the base of hairs 2 hairs (×) seem to have grown already while others are collapsed Amikacin treated animal 15 mg per kg body weight for 10 days (the recommended human dose) 10 days survival ×6600 *Fig 15* Outer hair cells of the 1st coil from base It shows clearly the early degeneration of single hairs (*arrows*) (Cf Fig 5) Gentamicin treated animal 150 mg per kg body weight for 10 days 22 days survival ×10800 *Fig 16* Final stage of hair degeneration after Amikacin treatment Single hairs are no longer discernible This form of hair degeneration was found predominantly after longer survival times Amikacin 300 mg per kg body weight for 10 days 22 days survival ×12000

DISCUSSION

Our findings show that standardization in animal experiments is helpful in comparing the ototoxic potency of various drugs We found quite an individual difference in the response to the toxic action in the organ of Corti after an attempt to standardize the experiments We are sure that the combination of methods as reported by Wersall et al (1973) will reveal far better the final mechanism of degeneration caused by ototoxic drugs In addition cochleograms as introduced by Engstrom et al (1964) and applied by Kohonen (1965) will provide answers to many questions regarding quantitative problems of sensory cell degeneration It was the purpose of our investigation to show that a rapid and quite appropriate method such as scanning electronmicroscopy could constitute a valuable tool in comparative morphological studies In a study of the recent literature we were unable to find a plausible answer to explain the probable

mechanism of degeneration of the outer hair cells, especially in the lower coils We think that there is a relation between general toxicity of Amikacin and direct hair cell damage The animals which showed severe damage in the sensory structures after 22 days' survival needed a long time to recover from Amikacin treatment before they were sacrificed on the 22nd day Some of them stopped growing or did not regain their weight In the other animals which tolerated the daily injections of Amikacin well, we found less pronounced damage in the outer hair cells All known theories fail to explain satisfactorily why mainly the outer rows of outer hair cells degenerate first in the lower coils, whereas hair cells of the 2nd and 3rd coils are less severely affected Scanning microscopy is certainly a useful tool with which to provide a preliminary view in questions which finally must be studied by various morphological methods

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ZUSAMMENFASSUNG

Es war das Ziel dieser Untersuchung mit Hilfe der Rasterelektronenmikroskopie im Tierversuch die Ototoxizität neuer Aminoglykosidantibiotika an Hand bekannter Schadigungsmuster zu untersuchen Meerschweinchen wurden für jeweils 10 Tage mit 2 s c Injektionen Amikacin behandelt Es wurden 15 75 150 300 mg/kg Körpergewicht und Tag entsprechend der empfohlenen Humandosis eingesetzt bzw 5 10 und 20fach uberdosiert Tobramycin und Gentamicin wurden in einfacher 10- und 50facher Uberdosierung eingesetzt entsprechend 3 30 und 150 mg/kg Körpergewicht

Die Tiere uberlebten 1 bzw 22 Tage Nach einem Tag Uberlebenszeit fanden wir bei den Tieren die mit 300 mg/kg Korpergewicht behandelt wurden entsprechend einer 20fachen Uberdosierung starkere Schadigungen an den Haarzellen als nach Tobramycin/Gentamicin Therapie die jeweils mit 150 mg also 50fach uberdosiert eingesetzt waren Nach 22 Tagen wiesen die mit Amikacin behandelten Tiere starke Schadigungen allerdings bei den einzelnen Tieren unterschiedlich ausgeprägt in allen Windungen auf wahrend die mit Gentamicin und Tobramycin behandelten Tiere starkste toxische Schäden vorwiegend in den unteren aufwiesen wobei gleichzeitig die zweite und dritte Windung fast normal erschien Verschiedene Formen von Stereozilienderegeneration wurden vorgefunden Fur das Amikacin fanden wir bereits bei Anwendung niedrigster Dosierungen Blahformen der Stereozilien

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METABOLIC DISORDER OF OTOCONIA AFTER STREPTOMYCIN INTOXICATION

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Abstract Little is known about the origin or the metabolism of otoconia Streptomycin sulfate was found to cause a decrease in their number on the otolithic membrane of the utricle and saccule in guinea pigs The remaining otoconia on the otolithic membrane varied in shape and size and giant otoconia with multilayered arrangement were observed by means of a scanning electron microscope These otoconia contained the normal amount of calcium in the form of standard calcium calcite crystal The lost otoconia were found attached to the surface of some vestibular dark cells Otoconia in this new position were irregular, shrunken or fragmented Their calcium content measured with an X ray micro-analyzer was variably diminished The dark cells appeared to be actively engaged in absorption of calcium ions from the attached otoconia The dark cell is considered to be a receptacle for the disposal of otoconia

The otoconia in man and other mammals consist of crystalline calcium carbonate in form of calcite (Carlström, 1963, Carlstrom & Engstrom, 1955) In an electron microscopic study, Lim (1973) has confirmed that mammalian otoconia are composed of an organic matrix and calcium carbonate which could be removed by decalcification In contrast to their well-established morphology and composition, little is known about their origin and metabolism

The ototoxic effect of streptomycin sulfate on the otolithic membrane and the sensory hairs was investigated on the utricle and saccule of guinea pigs The sensory hairs on the surface of sensory cells were found to be reduced in number This change was marked at the striola regions On the otolithic membrane, a similar decrease in the number of otoconia was observed The above mentioned changes were more pronounced at the utricle than at the saccule In this work, we tried to investigate the fate of the reduced otoconia Various parts of the vestibular labyrinth were examined by scanning electron microscope and by X-ray micro analyzer

METHODS

Non albino, adult guinea pigs with a 300 g average body weight were used Intraperitoneal streptomycin sulfate, 250 mg/kg body weight was administered every second day The dose was modified each time according to variations in weight of the animals The guinea pigs were divided into four groups and were sacrificed at 2, 4, 6 and 8 weeks from the beginning of the injections A fifth, control, group was also included The temporal bones were dissected quickly and immersed in 2% glutaraldehyde solution buffered at pH 7 4 with Millonig's solution for 24 hours The labyrinth was dissected in Millonig s solution and for post fixation was placed in 2% osmic acid solution for 2 hours Tissues were dehydrated in ascending grades of alcohol, then placed in iso-amyl acetate for 30 minutes The





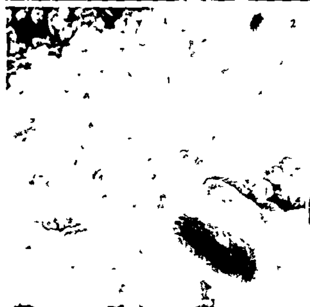




Fig. 1. Normal surface appearance of the guinea pig utricle.
Fig. 2. Normal otoconia on the otolithic membrane of the striola region.
Fig. 3. Layers of otoconia became thinner at the striola region of the utricle (*arrow*) after 4 weeks of streptomycin sulfate injection.
Fig. 4. Enlargement of striola region shown in Fig. 3. The disappearance of small otoconia is seen (*arrow*).

criticalpoint drying method using carbon dioxide was applied. The dried specimens were then coated with gold and the scanning electron microscope JSM-U3 was used. The JXA-50A X-ray micro-analyzer was used to determine the amounts of calcium in selected specimens.

RESULTS

In the macula of the utricle in the control group, the otoconia layers were thicker at the *pars externa*, while in the striola region they were thinner (Figs. 1, 2). On the other hand, in the macula of the saccule, the otoconia layers were thicker at the striola and thinner at the

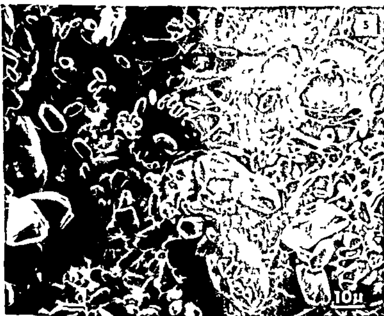




Fig 5 The remaining otoconia at the utricular otolithic membrane after streptomycin sulfate injection Some giant otoconia are seen Otoconia are partially embedded in the gelatinous layer (*arrow*)
Fig 6 The tip of a large otoconia showing multilayered arrangement

periphery The length of otoconia varied from 0 1 to 15 µm

In the 4-week injected group, the layers of otoconia became thin at the striola region, of both the utricle (Fig 3) and the saccule This change was more marked at the utricle, where only a few otoconia were left on the otolithic membrane of the striola This loss in the number of otoconia became more marked in the 6-week and 8 week injected groups (Figs 4, 5) The disappearance of otoconia could be due to loss of the supporting gelatinous substance secreted normally from the underlying supporting cells Variations in shape and size of the remaining otoconia over the otolithic membrane were also noticed Some large

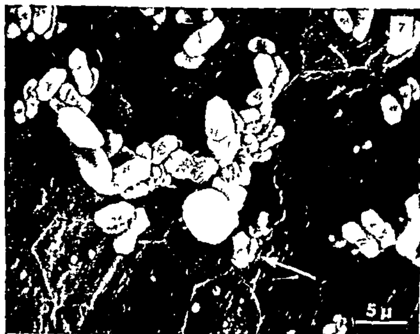
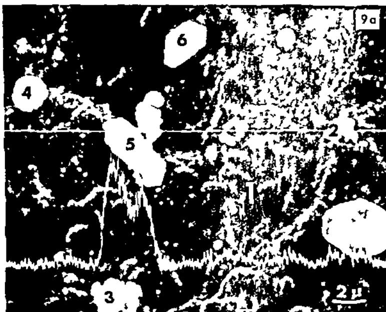
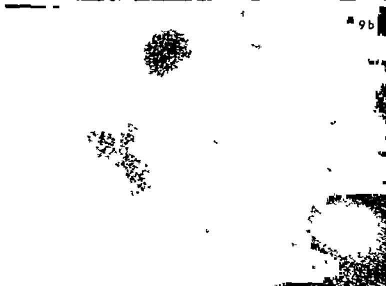




Fig 7 The dark cell area after streptomycin sulfate injection Otoconia are seen attached to the surface of cells Some otoconia are fragmented *(arrow)*
Fig 8 The otoconia attached to the dark cells Their surfaces appear granular with many pores Some otoconia are shrunken

otoconia measuring more than 15 μm in length were observed (Fig 6) The tips of these giant otoconia demonstrated a multilayered arrangement The amount of calcium in these otoconia made up about 40% of their contents This amount was equal to that present in the standardized calcium calcite crystal At the striola region where otoconia had disappeared only 1% calcium was found On the surface of some dark cells, numerous otoconia of varying shape and size were observed (Fig 7) The surface of these otoconia was irregular with many pores (Fig 8) Some appeared fragmented others were collapsed and shrunken The amount of calcium in the dark cell area, where otoconia were attached, was only 0 5% The amount of calcium in the otoconia attached to the dark cells varied according to





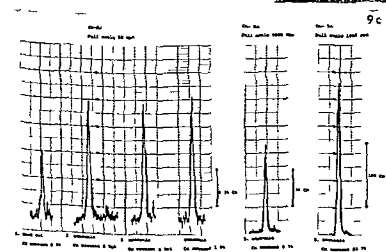


Fig 9 (*a*) The dark cell area and the attached otoconia which have been subjected to X ray micro-analysis The figures indicate the spots examined The line indicates the scanning line measured (*b*) Secondary X ray image of the above picture The large normal appearing otoconia (5 6) and the un numbered one in the right corner are shown Smaller or fragmented otoconia do not appear (c) The amounts of calcium measured by the spot to spot method in (*a*) are demonstrated Compare the size and shape of the otoconia seen in (*a*) and their calcium content in this graph The calcium contents at the different points are point 1 at dark cell surface 0 5% point 2 at fragmented otoconia 0 95% point 3 at fragmented otoconia 0 88% point 4 at small otoconia 1 0% point 5 at normal-appearing otoconia 6 7% point 6 at normal-appearing otoconia 23 7%

their morphology In the normal-appearing otoconia, the amount of calcium was 23 7%, the shrunken, deformed and fragmented otoconia showed smaller amounts (Fig 9) The surface of dark cells appeared to represent the area for disposal of otoconia In this position, otoconia were shrunken and fragmented and their calcium contents were reduced to varying degrees

DISCUSSION

The otoconia on the otolithic membranes are constantly renewing themselves So far, however, little is known about their origin and metabolism

When streptomycin was injected into guinea pigs, the otoconia on the otolithic membranes diminished as the amount of streptomycin increased, while giant otoconia appeared on the surface of the otolithic membrane The otoconia which had disappeared from the otolithic membrane were found attached on the vestibular dark cells This fact proves that streptomycin has some effect on sensory cells well as on otoconia

The giant otoconia could be the outcome of bnormal crystallization due to the toxic effect of streptomycin Similar large otoconia were demonstrated in the genetically pallid mouse which shows a specific otolith defect (Lim & Erway, 1974) The possibility of fragmentation of these large otoconia should be investigated as smaller otoconia with irregular shapes were also observed over the surface of dark cells At the regions where otoconia had disappeared, the surface of the otolithic membrane showed an amorphous ground substance with some holes in which the remaining otoconia were partially embedded

By using the scanning electron microscope and X-ray micro analyzer otoconia were observed attached to the dark cells The otoconia on the dark cells were found fragmented and shrunken to various shapes The calcium contents in the otoconia were diminished to varying degrees The surface of the dark cells appeared to be the site for disposal of otoconia

It has already been reported by Lim (1973) that the dark cells were concerned with the dissolution of otoconia, in normal guinea pigs cats, squirrels and monkeys Preston et al (1975) confirmed Lim's previous observation by using radioactive calcium

Upon injection of streptomycin, the attachment of otoconia to the surface of otolithic membranes became loosened This caused the otoconia to fall easily onto the surface of the dark cells It is easier to measure the contents of calcium in each otoconia by using the X ray micro analyzer The ultrastructure of the dark cells in the utricle and ampulla has been described earlier (Smith, 1956, Kimura et al 1974, Iurato, 1967, Kimura, 1969) Their morphological patterns were found to be similar to other cells concerned with ion transportation, such as in renal tubules (Rhodin, 1958), and choroid plexus (Pease, 1956) In the present study, the changes observed in the otoconia attached to the dark cells and the decrease in their calcium content indicated that calcium ions were actively absorbed by these dark cells This has clearly revealed part of the metabolism of otoconia The otoconia of the saccule were reduced in number after streptomycin injection, though not to the same extent as in the utricle It seems that this is caused by the saccule not having dark cells at the site in question

However, it is not yet known where the absorption of the otoconia takes place after their displacement from the saccule This is the problem still remaining It is assumed that some of the vestibular dark cells are concerned with the metabolism of saccular otoconia

ZUSAMMENFASSUNG

Uber Ursprung und Wandlungen der Otoconia ist wenig bekannt Streptomycin Sulfat bewirkt Verminderung ihrer Zahl an den Otolithmembranen von Sacculus und Utriculus im Meerschweinchen Die restlichen Otoconia an der Otolithmembran zeigten verschiedene Form und Größe

Riesenotoconia in vielschichtiger Anordnung wurden mit dem Raster Elektronenmikroskop beobachtet sie hatten einen normalen Inhalt an standardisierten Calcitkristallen Die restlichen Otoconia wurden an der Oberflache von vestibularen dunklen Zellen gesehen In dieser neuen Lage waren sie unregelmäßig geschrunken und zerbrockelt Ihr Calciuminhalt, gemessen mit einem Rontgen-Mikroanalysator, war in verschiedenen Graden vermindert Die dunklen Zellen schienen aktiv beschäftigt, Calcium von den anliegenden Otoconia absorbierend Die dunkle Zelle gilt als Stelle für die Ablagerung von Otoconia

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VESTIBULAR AND OPTOKINETIC RESPONSES OF THE WHITE CAT

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Abstract The vestibular and optokinetic responses of a group of white cats were evaluated and compared with the responses from a control group of pigmented animals The results indicate that in all cases the white cats exhibited varying degrees of vestibular and/or optokinetic dysfunction which in some animals varied from test session to test session

Histological descriptions of the inner ear of the congenitally deaf white cat (Bosher & Hallpike, 1965, 67, West & Harrison, 1973, Wilson [illegible] Kane, 1959, Wolff, 1942) indicate the [illegible]y elements of the cochlea and saccule [illegible] within the first few weeks of post[illegible] life, whereas the utricle and semicircular canals continue to develop in a normal fashion

Although numerous studies have concerned themselves with the auditory function of the white cat (reviewed by West & Harrison, 1973), this investigation is the sole report on the vestibular and optokinetic responses of these animals

METHODS

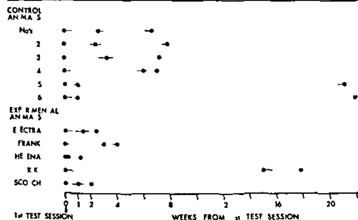
Each cat, under ketamine anesthesia (Ketajet Bristol Laboratories, 15 mg/kg, i m), had its tympanic membranes examined and its canine teeth drilled for subsequent retraining measures (Henriksson et al 1961) After a minimum of 3 days, each cat was observed as to its gait and its ability to right itself from th[e] left and right ear down, and supine position[s] (in light and then blindfolded) After being re[-]strained, needle electrodes were placed at th[e] outer canthus of each eye, with a referenc[e] electrode in the neck musculature Ey[e] movements resulting from optokinetic stimula[-]tion, administered by a rotating striped drum positional tests (performed in light and again in total darkness), and bilateral bithermal (46 and 29°C) calorics were rectilinearly recorde[d] on a polygraph machine with a time constan[t] of 11 sec Caloric irrigations were performe[d] in moderate light for 40 sec at which time th[e] irrigation was discontinued The room was then totally darkened and the resulting vestibulo-ocular responses recorded A rest interval of 5 min separated each caloric test

Three testing sessions at random intervals (see Table I) comprised a complete testing se[-]quence For the purposes of determining what effects, if any, this testing paradigm had on normal vestibular responses, a group of 6 pigmented cats served as controls

In order to provide a group of 5 experimen[-]tal animals for this investigation, a total of 10 cats were selected at random from a colony of white cats Three of the 10 cats were found to have broken canines and were therefore not tested Of the remaining cats, 2 broke their canines before the entire testing sequence was completed The results from this latter group of animals are not included in this report

Supported by U S P H Grant No NS 10522

Table I *Test intervals for control and experimental animals referenced from the first test session*



RESULTS

A *Control Animals*

The 6 cats comprising the control group of animals showed little intersession variability in their vestibular or optokinetic responses When present these differences could either be ascribed to variations in the observer s subjective interpretation of the response (gait and righting tests) or to a qualitative change in the total response pattern i e the intensity of the caloric response may have changed from one test session to another however the relative differences between labyrinths if present remained constant

B *Experimental Animals*

(see Table II for summary)

(1) *Electra*

A 9 year-old totally white female cat with bilaterally blue eyes Observations during the first test session indicated a normal gait with normal righting reflexes in all positions except from the supine when blindfolded In this situation Electra was observed to falter slightly to the right after landing Optokinetic responses were normal and no spontaneous or positional nystagmus was recorded Results from caloric examination indicated a mild left canal paresis (Fig 1) Nine days later Electra was tested for a second time and observed to have a normal gait with righting reflexes not being performed because of difficulty in handling the animal Optokinetic responses were normal and no spontaneous or positional nystagmus was recorded Results from caloric examination indicated a moderate left canal paresis (Fig 2) Five days later Electra was tested for the third time and observed to have a normal gait and normal righting reflexes Optokinetic responses were normal and no spontaneous or positional nystagmus was recorded Results from the caloric examination indicated a moderate right canal paresis was present (Fig 3)

(2) *Frank*

A 5 year-old totally white male cat with bilaterally blue eyes Observations during the first test session were not made as to gait Righting reflexes and optokinetic responses were found to be normal No spontaneous or positional nystagmus was recorded in the light However in the dark a slow right beating nystagmus was observed with the animal in the prone position (Fig 4) There was no nystagmic response recorded from the other positional orientations Results from caloric examination indicated a left directional preponderance (Fig 5) Three weeks later Frank was tested for the second time His gait was observed to be normal as were the righting reflexes from the right and left ear down posi

Table II. *Summary of white cat vestibular and optokinetic responses*

N, Normal, AB, Abnormal, NO, Not observed, *, See text for detailed description, R,L,P,S, Animal orientations of r ear down, left ear down, prone, and supine respectively, (R),(L), Indicates direction of fast component of nystag response Cross hatched boxes are tests performed in total darkness or with the animal blindfolded (see Methods)

CAT NAME	TEST SESSION	DAYS FROM FIRST SESSION	GAIT	RIGHTING REFLEXES	OPTOKINETICS	SPONTANEOUS OR POSITIONAL NYSTAGMUS	CALORIC RESPONSES
ELECTRA	1	0	N	N S	N	none none	Mild left canal paresis
	2	9	N	no no	N	none none	Moderate left canal paresis
	3	14	N	N N	N	none none	Moderate right canal paresi
FRANK	1	0	NO	N N	N	none P(R)	Left directional preponder
	2	21	N	S S	N	none none	Moderate left canal paresis
	3	26	N	N S	N	none abnormal*	Moderate left canal paresis
HELENA	1	0	N	R,S L	N	none R(R),S(R)	Strong left canal paresis
	2	1	N	S R,L	N	none P(R)	Abnormal left canal respons
	3	8	N	N R,L,S	N	none none	Moderate right canal paresi
KIKI	1	0	N	N N	AB*	none none	Normal
	2	104	N	N R	AB*	none none	Normal
	3	125	NO	N N	AB*	none none	Normal (warm water only)
SCOTCH	1	0	NO	L,S R,S	N	abnormal* abnormal*	None
	2	7	NO	P L,S R,L,S	N	none none	Slight vestibular response*
	3	12	NO	L,S L,S	N	abnormal* abnormal*	Slight vestibular response*

tions When righting from the supine, in both the light and blindfolded conditions, Frank faltered on the turn and landed broadbased with some degree of unsteadiness Optokinetic responses were normal and no spontaneous or positional nystagmus was recorded Results from caloric examination indicated a moderate left canal paresis (Fig 6) Five days later, Frank was tested for a third time His gait was normal and the only abnormal righting reflex was observed from the supine with the animal blindfolded Optokinetic responses were normal, and no spontaneous or positional nystagmus was recorded when the animal was positioned in the light In the dark, however, mild right-beating nystagmus with no appre able latency and which was not sustained w provoked after reorientation to the prone po tion from the right and left ear down, a supine orientations Results from caloric e amination indicated a moderate left can paresis, similar to that found during the seco test session

(3) "Helena"

An 8-year-old totally white female cat with blue left iris and a pigmented right iris Obse vations during the first test session indicated

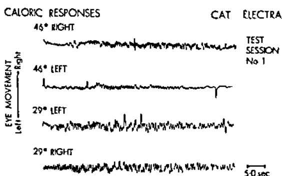


Fig 1 Cat "Electra" test session 1 Polygraph tracings of eye movements resulting from caloric examination Direction of eye movement and time scale as indicated

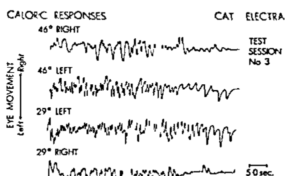


Fig 3 Cat "Electra", test session 3 Polygraph tracings of eye movements resulting from caloric examination Direction of eye movement and time scale as indicated

normal gait Abnormal righting reflexes in the light were observed from the right ear down and supine positions, and from the left ear down position when blindfolded Optokinetic responses were normal, as were the positional tests in the light In the dark, however, a right beating nystagmus with a latency of 5 sec and lasting approximately 25 sec was elicited from the right ear down position (Fig 7) A similar response was recorded when the animal was placed in the supine position (Fig 7) Results from the caloric examination indicated a strong left canal paresis (Fig 8) One day later, Helena was tested for the second time and was observed to have a normal gait In the light, righting reflexes were normal from the right and left ear down positions From the supine, however, a slight underturn was observed When the animal was blindfolded, abnormal righting reflexes were observed from both ear down positions Optokinetic responses were normal, and the only abnormal ocular responses recorded during positional testing were found in the dark with the animal prone This response consisted of a slow right beating nystagmus of moderate amplitude which had no latency and was sustained for approximately 35 sec (Fig 7) Results from caloric examination revealed an abnormal left ear response from both warm and cold irrigations (Fig 9) These consisted of alternating saccades with little indication of the presence of a slow component Although the response from the right ear appeared normal there was a marked asymmetry between responses to warm and cold irrigations One week later, Helena was tested for the third time Her gait appeared normal as did righting reflexes in the

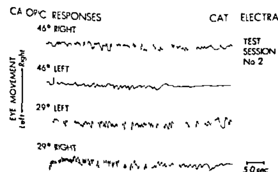


Fig 2 Cat "Electra", test session 2 Polygraph tracings of eye movements resulting from caloric examination Direction of eye movement and time scale as indicated

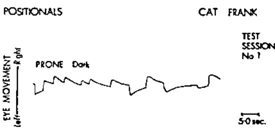


Fig 4 Cat "Frank" test session 1 Polygraph tracings of eye movements resulting from animal being placed in the prone position in the dark Direction of eye movement and time scale as indicated

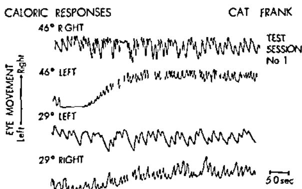
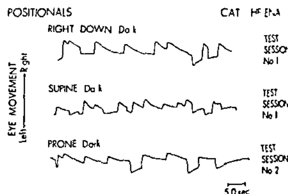


Fig 5 Cat Frank test session 1 Polygraph tracings of eye movements resulting from caloric examination Direction of eye movement and time scale as indicated



the dark Third tracing is for test session 2 with the anima prone in the dark Direction of eye movement and tim scale as indicated

light In the dark, however, abnormal landings were found from all tested positions Optokinetic responses were normal and no spontaneous or positional nystagmus was recorded Results from caloric examination indicated a moderate right canal paresis (Fig 10), with a short episode of saccadic movements in the initial portion of the cold left response

(4) *"Kiki'*

A 3 year-old totally white female cat with ilaterally blue eyes During her first test ses Kiki was observed to have normal gait normal righting reflexes Abnormal optokinetic responses during clockwise drum rotation was recorded (Fig 11) These consisted of left nystagmic eye movements interrupted by saccades to the right Normal optokinetic responses were recorded for counterclockwise stimulation (Fig 11) No spontaneous or positional nystagmus was recorded Results from the caloric examination did not indicate any abnormality Approximately 15 weeks later, Kiki was tested for the second time and observed to have a normal gait Normal righting reflexes were observed from all positions except from the right ear down when blindfolded Optokinetic responses were abnormal for counterclockwise stimulation because of the presence of interspersed saccades to the right (Fig 11) No spontaneous or positional nystagmus was recorded Results from the caloric examination did not indicate any abnormality Three weeks later Kiki was tested for the third time

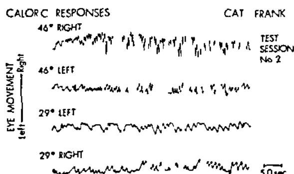


Fig 6 Cat Frank, test session 2 Polygraph tracings of eye movements resulting from caloric examination Direction of eye movement and time scale as indicated

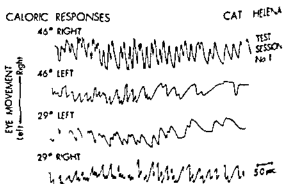


Fig 8 Cat Helena test session 1 Polygraph tracings of eye movements resulting from caloric examination Direction of eye movement and time scale as indicated

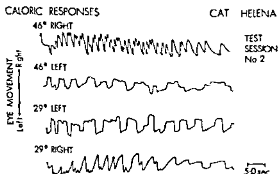


Fig 9 Cat "Helena', test session 2 Polygraph tracings of eye movements resulting from caloric examination Direction of eye movement and time scale as indicated

Although gait was not observed righting reflexes were normal Abnormal optokinetic responses similar to those occurring during the second test session were recorded No spontaneous or positional nystagmus was recorded Warm water caloric responses were normal and equal bilaterally, even to the extent of a secondary nystagmus occurring 40 sec after cessation of the irrigation (Fig 12) Cold water irrigations were not adequately performed and therefore results were not considered

(5) "*Scotch*"

A 10-year-old totally white male cat with bilaterally blue eyes His gait was not observed during the first test session In the light, righting reflexes were abnormal from the left ear

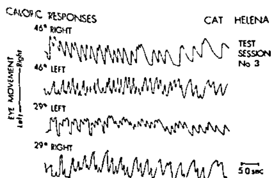


Fig 10 Cat "Helena', test session 3 Polygraph tracings of eye movements resulting from caloric examination Direction of eye movement and time scale as indicated

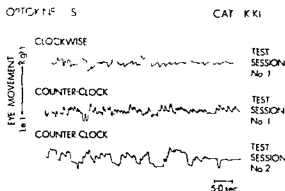


Fig 11 Cat 'Kiki', test session 1 Eye movement responses to clockwise (tracing 1) and counterclockwise (tracing 2) optokinetic stimulation Third tracing is the response to counterclockwise optokinetic stimulation during the test session 2 Direction of eye movement and time scale as indicated

down and supine positions When blindfolded he had difficulty in righting from the right ear down but not the left ear down position Scotch did not right himself from the supine Optokinetic responses were normal In the prone position, with lights on, Scotch had a low amplitude, slow frequency (approximately 0 8 Hz) square to pendular form of ocular oscillation (Fig 13) This appeared to be a dynamic response as characteristics of these eye movements would change suddenly Changes in the frequency, amplitude, and/or waveform duration were recorded when the animal was in the right ear down, left ear down, and supine positions The amplitude diminished significantly when testing was performed in total darkness except for the supine where the amplitude slightly increases

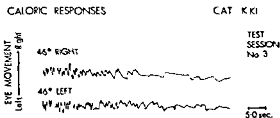


Fig 12 Cat "Kiki" test session 3 Polygraph tracing of eye movements resulting from caloric examination (warm water only) Direction of eye movement and time scale as indicated

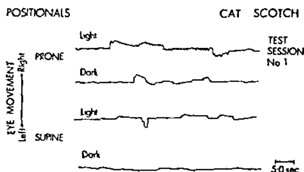


Fig 13 Cat Scotch test session 1 Eye movement responses to position tests with the animal prone and supine in both light and dark conditions Ocular oscilla tions as described in the test are evident from the trac ings Direction of eye movement and time scale as indi cated

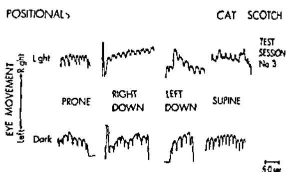


Fig 15 Cat Scotch test session 3 Eye movement re sponses (at 10×normal gain) occurring in the prone rig and left ear down and supine positions in both light a dark conditions Direction of eye movement and tir scale is indicated

(Fig 13) When placed in position for irriga tion i e , 60° inclined from the horizontal with the room totally dark, and with the amplifica tion at ten times normal gain (0 05 mV/cm), a pendular type of ocular oscillation was re corded when the lights were off This changed to a rhythmical nystagmic like response when the lights were again turned on (Fig 14) Small amplitude ocular oscillations were recorded during the irrigation of the right ear but not the left ear No nystagmic responses to any of the ... were recorded One week later, ... was tested for the second time and ...ed to have normal gait but abnormal righting reflexes He had difficulty maintaining an upright stance after being dropped from the right ear down and supine positions No right ing ability was observed from the left ear down

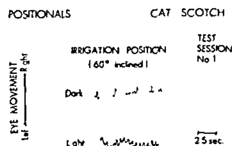


Fig 14 Cat Scotch" test session 1 Eye movement re sponses (at 10×normal gain) occurring when the animal is placed in position for caloric irrigation (60° inclined from horizontal) in both dark and light cond tions Direction of eye movement and time scale as indicated

position When blindfolded, a slight unstead ness was observed after righting from the righ ear down position, with no righting abilit whatsoever from the other orientations N spontaneous or positional nystagmus nor an type of oscillatory eye movement was re corded Results from the caloric examinatio revealed an extremely weak nystagmus Dur ing the irrigations of the right ear, oscillator eye movements were provoked which cease at the end of the irrigation with removal of th irrigation tube from the external canal Thi was not seen for the left ear The third an final test session was 5 days later The gait o Scotch was not observed Righting reflexes i the light were normal from the right ear dow position only Scotch had some difficulty right ing from the left ear down position and did no seem to actively right himself when dropped from the supine These same results were enhanced when Scotch was blindfolded Optokinetic responses were normal During positional tests, ocular oscillation were re corded at 10×normal gain (Fig 15) which ap peared similar to those present during Scotch s first test session Characteristics of the ocular oscillations changed with a change in position or test condition Caloric responses were ex tremely mild Ocular oscillations were present during the entire warm water left ear response at times during warm water irrigation of the right ear, but were totally absent during either of the cold water irrigations

DISCUSSION

In this study, the prominent finding is the presence of altered vestibular and/or optokinetic responses in all of the white cats tested. The control experiments with pigmented cats indicate these altered responses are, in fact, a characteristic of the white cat, and not the result of the testing paradigm. A summary of the experimental cat data is presented in Table II. It indicates (1) abnormal righting reflexes were observed at times in *all* cats, (2) abnormal optokinetic responses were recorded from one cat (Kiki), (3) episodes of spontaneous or positional nystagmus were recorded from 3 cats (Frank, Helena, and Scotch), and (4) abnormal vestibulo-ocular responses to caloric examination were found in every cat except one (Kiki). Intersession variabilities of these responses are also evident from Table II.

Regarding the abnormal saccadic movements of Scotch, electrical artifact was considered as a factor, but was concluded to be unlikely in that (1) recordings from all animals were made in the same manner using the same recording system, yet only Scotch exhibited this specific form of ocular oscillation, and (2) qualitative changes were recorded in the character of the saccadic responses during those testing sessions when they were present.

Marcus (1968) presents the only description of the vestibular responses of the Waadenburg individual to which the white cat has been likened. His study examined 53 members of a four generation family exhibiting degrees of Waadenburgism, with 22 of these family members available for vestibular evaluation. It is interesting that Marcus found "Labyrinthine involvement is widespread throughout most of the members of this family regardless of the other features of the syndrome". Continuing . . . the vestibular malfunction was the most prominent feature, more so than the sensorineural hearing loss, and appeared in a number of the children who had normal hearing and no other outward signs of the syndrome".

In summary, a group of animals from a colony of white cats were serially tested (three times each) using conventional vestibular techniques and were found, in all cases, to exhibit varying degrees of vestibular and/or optokinetic dysfunction, which in some animals, varied from test session to test session.

ZUSAMMENFASSUNG

[illegible] weißen Katzen einen wechselnden Grad vestibularer und/oder optokinetischer Storungen die in manchen Tieren von Untersuchung zu Untersuchung schwankten

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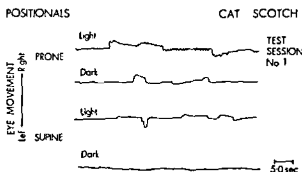


Fig 13 Cat Scotch test session 1 Eye movement responses to position tests with the animal prone and supine in both light and dark conditions Ocular oscillations as described in the test are evident from the tracings Direction of eye movement and time scale as indicated

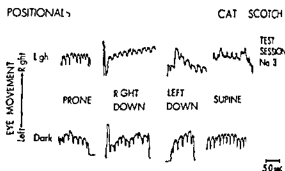


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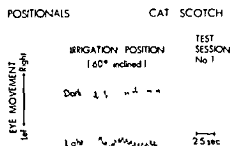


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ZUSAMMENFASSUNG

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FINE STRUCTURE OF THE MEDIAL AND DESCENDING VESTIBULAR NUCLEI IN NORMAL RATS AND AFTER UNILATERAL TRANSECTION OF THE VESTIBULAR NERVE

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Abstract Cells and neuropil are of similar structure in the descending and medial vestibular nuclei Two cell types were found small neurons and larger cells Three types of axon terminals have been defined small and large terminals containing spherical vesicles (SV) and terminals with elongated vesicles (EV) Small SV terminals contact perikarya and dendrites whereas large SV terminals contact fine dendritic branches EV terminals contact neurons at perikarya and large as well as small dendritic profiles SV terminals can be presynaptic to EV terminals Vestibular nerve transection resulted in degeneration of small SV terminals which were found from the first to fifth day in the ipsilateral nuclei Glycogen [granu]les were found in various types of axon terminals [bi]laterally in the vestibular nuclei 3–5 days after right vestibular transection They were not observed in normal animals or in degenerating vestibular afferent terminals

A great volume of literature is available on the morphology and fiber connections of the vestibular nuclei at the light microscopic level (cf Brodal 1974 for a recent review) The major fiber contingents and their termination areas are consequently well known Functional interpretation of these connections is aided by descriptions of synaptic contacts which depend on the use of the electronmicroscope The necessary ultrastructural studies have in the past been concentrated on the lateral nucleus of Deiters (Mugnaini & W[al]berg, 1967, Mugnaini et al , 1967*a* *b*, Sotel[o &] *Palay, 1968, 1970)* which, originating from [the] basal lamina constitutes the phylogenetic[ally] least original part of the vestibular comp[lex] (Vraa & Jensen 1956) This study extends [the] electronmicroscopic description to include [the] more original medial and descending ve[sti]bular nuclei which are derived from the [alar] plate (Vraa & Jensen, 1956)

The border zone between medial and [de]scending nuclei was chosen not only beca[use] it can be easily recognized and excised [for] electronmicroscopic examination but also [be]cause it seems to represent one function[al] unit receiving common primary vestibu[lar] (Walberg et al , 1958, Stein & Carpenter 19[67] Gacek 1969) and cerebellar (Angaut & Brod[al] 1967) as well as polysynaptic spinal affere[nts] (Fredrickson et al , 1966) The border betwe[en] both nuclei is defined by the presence of lon[gi]tudinal fiber bundles in the descending nucle[us] which are largely composed of Deitero-spi[nal] fibers passing this nucleus on their way to t[he] spinal cord (Brodal et al , 1962) Neurons [on] either side are similar in appearance and the[ir] dendrites cross the border from both sid[es] (Haughie Hanssen 1968) Since there is th[us] no functional reason to assume that the rece[...]

This study was supported by a stipendium to D Schwarz from the Max Planck Gesellschaft and by the Medical Research Council of Canada

nized nuclear border separates two differing neuron groups, the transition area is treated as one unit in this study

MATERIALS AND METHODS

Observations were made in 12 rats of a vital mixed breed between albino and gray Under Pentobarbital anesthesia and aseptic precautions the vestibular nerve of 10 rats was exposed via a transutricular approach and transected The vestibule was then filled with gelfoam and the wound closed Following the operation the animal's motor performance was observed during recovery from anesthesia and then daily until sacrifice Forced circling towards the lesioned side around the longitudinal axis during light anesthesia was observed, and circular locomotion of the awake animal occurred up to 6 days postoperatively Two rats were sacrificed after survival times of 1, 2, 3, 4 and 6 days each Two unoperated rats served as controls

Under Nembutal anesthesia and during artificial respiration the animals were perfused through the ascending aorta for 30 minutes with 2 5% glutaraldehyde in phosphate buffer at pH 7 3 The brainstem was quickly removed and sectioned horizontally, less than 1 mm thick These slices were kept in the fixative for one hour at 4°C, then washed in cold buffer and transferred to 1% OsO_4 in Veronal buffer until the osmium stain permitted identification under a dissection microscope of the medial and descending vestibular nuclei containing the characteristic longitudinal fiber bundles Two or three small tissue blocks were excised at the border between the descending and medial vestibular nucleus bilaterally and kept in 1% OsO_4 in Veronal buffer for one hour at 4°C The tissue was then dehydrated stained en bloc in a saturated solution of uranyl acetate in absolute acetone (Westrum 1965) and embedded in Epon Ultrathin sections were cut with glass knives on LKB or Porter Blum ultramicrotomes and examined in a Siemens Elmiscop I or a Philips 300 electronmicroscope

RESULTS

A *Normal Structure*

In this part the transition zone between the medial and descending vestibular nuclei from both sides of the two unoperated rats and from the left side of the 10 rats with right vestibular nerve transection is described

Postsynaptic structures Two types of nerve cells were found in both nuclei

(1) Small neurons (Fig 1) had a narrow cytoplasmatic rim surrounding a relatively large nucleus In the cytoplasm all typical neuronal organelles were present, Golgi zone and rough endoplasmic reticulum being relatively poorly developed

(2) Large neurons (medium sized cells of cytoarchitecture, *Brodal & Pompeiano, 1957*) were rich in cytoplasm with a well developed Golgi zone and rough endoplasmic reticulum They commonly contained many dense inclusions (possibly lysosomes), multivesicular bodies and dense core vesicles of varying size

Cytoplasmic processes invaginate deeply into the nucleus of both cell types (Fig 1) These invaginations are usually densely filled with free ribosomes Astroglial processes contact most of the perikaryal surface mostly in form of thin astroglial sheaths Dendrites and myelinated axons were found in direct contact with the perikaryal membrane Solitary terminal boutons containing round or elongated synaptic vesicles synapse with somatic membranes Occasionally two or three boutons contacting the soma are adjacent, but axo-axonic synapses between them were not observed

Larger perikarya tended to have more synaptic contacts per cell membrane area than smaller neurons

The greatest density of synaptic terminals per neuronal membrane surface is found on









Fig 5. A SV terminal synapsing at a dendritic pit and an EV terminal. The shape of these vesicle may be discoid.

Fig. 6. A small SV terminal with its myelinated axon Note subjunctional bodies.

Fig 7 Degenerating axo-somatic terminal on fir operative day

Fig 8 Degenerating axo-dendritic terminal on thi operative day

terminals with the largest round profile diameter approximately equal to the smallest diameter of the elongated vesicle, the true shape may be cylindrical (Fig 2 upper half)

Most small SV terminals appear to be end terminals which leave the myelin sheaths relatively close to synaptic contacts (Fig 6) 'En passant' terminals budding off a node of Ranvier have also been found

Single, dense core vesicles were seen in all possible types of SV and EV terminals (Fig 4), but also within perikarya and myelinated axons Coated (complex, alveolate) round vesicles could be found in SV and EV terminals (Fig 4) Rarely was the lumen of a coated vesicle noted to be contiguous with the extracellular space

The large SV terminals and the EV terminals may be lobated and contact several dendrites and sometimes a perikaryon with synapses

Distribution of axon terminals

Small SV terminals contact predominantly perikarya and large dendritic profiles, whereas the large SV terminals are almost exclusively seen in contact with dendrites, usually of smaller diameter EV terminals have been observed at all neuron partitions, at perikarya they are frequently adjacent to a SV terminal and both can have a common cover of one or several astroglial sheaths Smaller dendrites are frequently contacted at one side by a SV terminal and at the opposite side by an EV terminal and all three structures are entwined by a common layer of one or several astroglia processes

A consistent type of synaptic glomerulus could not be defined

Synaptic contacts

In the cerebral cortex a correlation between SV terminals and asymmetric synapses and between EV terminals and symmetric synapses has been described (Colonnier, 1968) Such a correlation has not been consistently verified for other brain regions including the lateral vestibular nucleus of the cat (Mugnaini et al, 1967*a*) Our observations support such a correlation for the medial and descending vestibular nuclei EV terminals contact post synaptic structures only with symmetric synapses, invariably there is very little osmiophilic material attached to either side of the two contacting cytomembranes Attachment of dense osmiophilic material to the post synaptic membrane has been found in connection with SV terminals (Figs 2, 3, 6), however, the amount of dense material can occasionally be scanty

At the presynaptic site of asymmetric synapses presynaptic dense projections (Gray, 1963) are frequently seen (Fig 3) Postjunctional dense bodies (Milhaud & Pappas, 1966) occur postsynaptically to large and small SV terminals (Figs 2, 3, 6) They have not been found adjacent to EV terminals Flat cisternes of smooth endoplasmic reticulum were frequently seen below synaptic sites (Fig 3) Gap junctions or tight junctions noted between large terminals and dendrites in the rat's lateral vestibular nucleus (Sotelo & Palay, 1970) were not found in the nuclei studied in this investigation despite a careful search

Axo axonal synapses have been occasionally seen small or large SV terminals can be presynaptic to EV terminals, however, sub synaptic SV terminals have never been observed Desmosomes were frequently found close to synapses, they were also seen between two adjacent terminals which were not connected by synapses

B Observations after Transection of the Right Vestibular Nerve

Degenerating axon terminals were observed after all survival times investigated, they were invariably small and bouton shaped or slender, contained spherical vesicles and were devoid of neurofilaments, they can therefore be classified as the small SV terminal subgroup described above Also the number of non-degenerating small SV terminals devoid of







Fig. 9. Microglia process contacting a perikaryon. The inclusion is interpreted as degenerated terminal after phagocytosis on third postoperative day.

Fig. 10. Axo-dendritic contact, contralateral, fourth day postoperatively.

Fig. 11. EV terminal, contralateral, fourth day postoperatively.

neurofilaments appeared to be greatly reduced.

Degeneration was of the primary dark type (Mugnaini & Walberg, 1967; Mugnaini et al., 1967*b*). The vesicle diameters in degenerating terminals were of greater variety than those noted in non-degenerating SV terminals: the number of enlarged vesicles appeared greater than that of unusually small ones (Cuénod et al., 1970, 1972; Kawana et al., 1971; Smith et al., 1966; Szentágothai et al., 1966). When synaptic contacts of these terminals could be identified the subsynaptic structure was either a perikaryon (Fig. 7) or a large dendritic stem (Fig. 8). This early type of degeneration was observed up to the fourth postoperative day but it was most frequently seen on the first and second day. Synapses were of the asymmetric type (Gray's type I) with a prominent subsynaptic osmiophilic layer (Figs. 7, 8).

At the third to fourth postoperative day most degenerating terminals no longer contained clearly outlined vesicles. During this time microglial processes (Mori & Leblond, 1969*a*) were frequently seen adjacent to degenerating terminals. Such processes often contained large dark inclusions with remnants of vesicles and mitochondria (Fig. 9). During the third to sixth postoperative day there was an impressive proliferation of microglia cells

which at the sixth day was the main sign of degeneration They often contained a complex system of ballooning smooth endoplasmic reticulum and large vesicular inclusions with relatively homogeneous contents of different osmiophilia

Proliferation of astroglial cells was also noted from the third to the sixth postoperative day as described by Mori & Leblond (1969*b*)

The degeneration of axon terminals and glia reaction was only seen ipsilateral to the transected vestibular nerve

When most primary darkened degenerating terminals were already phagocytized by glia terminals on both sides of the brainstem showed a reaction of microfilament hyperplasia These terminals belonged to either the large SV terminal group or the EV terminal group Both groups do not degenerate after vestibular nerve transection and normally contain comparatively few microfilaments

Another interesting finding after right vestibular nerve transsection was the occurrence of glycogen granules in various axon terminals of both sides In the two unoperated rats glycogen granules have not been observed in axon terminals, nor have they been found in degenerating vestibular afferent terminals

Fig 10 shows a SV terminal contacting a dendrite with an asymmetric synapse Glycogen particles are suspended between the vesicles This terminal could belong to the small SV terminal subgroup which includes the vestibular afferents The tissue sample was taken from the side contralateral to the transsected vestibular nerve

Large SV terminals containing microfilaments and contacting fine dendritic branches have also been found on both sides to accumulate glycogen particles

In Fig 11 an EV terminal is shown containing glycogen particles Glycogen has been observed in the different terminal types after each survival period studied Most frequently it was found ipsi and contralaterally in animals with survival times of 4 days The fourth day also appears to be a critical time in the course of central compensation for unilateral vestibular loss the rats walked in narrow circles during the first few days postoperatively, but starting on the third to fifth day their ability of relatively normal explorative locomotion was regained

DISCUSSION

This study extends histological knowledge concerning the border zone between the medial and descending vestibular nuclei into the ultrastructural level It is confirmed that cell types are similar on either side of the border which has been classically defined to separate these two nuclei (Brodal & Pompeiano 1957) Two neuron types seem to differ not only in size, but also by their synaptic contacts The larger neurons (medium sized cells of cytoarchitecture) had many axosomatic contacts which were rare at small neurons The neuropil was of similar appearance on both sides of the border, save for the large axonal bundles passing through the descending nucleus

The recognition of three classes of axon terminals does not imply that this nuclear area receives afferents from only three sources functionally different afferents may have a similar structural appearance Some terminals must originate from cells within these two nuclei themselves (Haughie Hanssen, 1968) It is therefore necessary to identify afferents experimentally, which was done in this study only for primary vestibular fibers These afferents belong in the class of the small SV terminals and exhibit a similar type of degeneration as described for the feline lateral vestibular nucleus (Mugnaini et al , 1967*b*)

The synaptic activation mediated by these vestibular afferents must be of great influence on the cells spike generation mechanism, since many degenerating terminals were found to contact perikarya and large proximal dendrites This is reflected in the short rise time of EPSPs in physiological experiments (cf Precht 1974) The fact that small SV ter-

minals without signs of degeneration can be found after complete section of the vestibular nerve does suggest, but not prove, that other afferents may belong to this class Also small extensions of the large SV terminals appearing in the section under examination may be indistinguishable from small SV terminals, particularly when no microfilaments appear in the section No reasonable suggestion can be made regarding the origin of large SV terminals

EV terminals may, at least partly, originate from cerebellar Purkinje cells which are known to send their inhibitory axons (Ito & Yoshida, 1964, Ito et al , 1964) into this region (Angaut & Brodal, 1967) According to Uchizono (1967) inhibitory terminals contain elongated vesicles and it is probable that at least one type of the elongated vesicles seen in this study reflects this cerebellar projection from the archicerebellum (Angaut & Brodal, 1967) It may be suggested that, depending upon fixation, elongation of vesicles may appear as artifacts for most terminal groups (cf Bodian, 1970) However, when the fixa ion method is kept constant, in particular the ity of an aldehyde fixative as in the study, different vesicle shapes can be ken to indicate different terminal systems (Valdivia, 1971)

A striking observation in this study was the presence of glycogen in non-degenerating axon terminals during the time of compensation after unilateral vestibular nerve section Elevation of brain glycogen has been reported in response to a variety of insults (mechanical damage, irradiation viral infection, etc cf Ibrahim, 1975 for a review) and the glycogen seen might therefore simply represent a nonspecific response Such an interpretation is probably not justified since nonspecific glycogen accumulation has been observed mainly in astroglia (Ibrahim 1975) Few reports are available on glycogen in axon terminals of mammals although it is commonly found in poikilothermic animals Glycogen is generally not seen in 'normal' terminals (Gray 1963 Walberg, 1966), however, it has been occasionally seen in degenerating axon terminals (Szentágothai et al , 1966, Smith et al , 1966 Berger, 1971, Lund, 1969) Degenerating vestibular terminals of this study did not contain glycogen Terminal glycogen should probably not be interpreted as a sign of cell death but rather as an indication of temporarily altered metabolism since it has also been observed when the fast axoplasmic flow was reversibly interrupted by colchicine (Cuenod et al , 1972) Glycogen in mammalian terminals has also been seen during critical stages of development Vaughn (1971) saw glycogen in boutons of the rat's spinal cord a few days before parturition and during the first postnatal week Lund & Lund (1972) found it in optic tract terminals within the tectum of rats only during the period when the young animals opened their eyes Glycogen stores can provide cellular elements with energy metabolism for periods of time when the blood supply of glucose and oxygen is insufficient The storage in terminals may therefore represent a protective mechanism in growing neural processes during the establishment of new or alteration of old synaptic contacts, the glycogen metabolism becoming unnecessary (and uneconomical) when the terminal has established its regular supply in its new environment It is reasonable to expect alterations of synaptic contacts in the ipsi and contralateral vestibular nuclei during the period of compensation from unilateral vestibular loss Our observations of glycogen in various terminals during compensation may thus be compatible with the previous reports on glycogen within mammalian axon terminals

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ZUSAMMENFASSUNG

Zellen und Neuropil sind von ähnlicher Struktur in Nucleus vestibularis descendens und medialis Es fand

sich zwei Zelltypen kleine Neurone mit engem Zytoplasmarand und mittelgroße Zellen mit reichlich Zytoplasma Drei Arten von Axonendigungen sind unterscheidbar kleine und große Endigungen mit runden Vesikeln und Endigungen mit ovalen Vesikeln Kleine Endigungen mit runden Vesikeln kontaktieren Perikarya und Dendriten, wahrend Synapsen mit großen Endigungen dieser Gruppe nur an feinen Dendritenästen gefunden wurden Endigungen mit ovalen Vesikeln kontaktieren die Neurone an Perikarya und Dedriten Endigungen mit runden Vesikeln konnen präsynaptisch zu Endigungen mit ovalen Vesikeln sein Nach Durchtrennung eines Vestibularisnerven degenerierten nur kleine Endigungen mit runden Vesikeln ipsilateral In verschiedenen Endigungstypen bilateral fanden sich drei bis fünf Tage nach dieser Operation Glycogen-Granula, die in normalen Tieren und in degenerierenden vestibularen Terminalen nicht gefunden wurden

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NORMAL VALUES IN A ROUTINE ENG TEST

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Abstract A total of 50 normal subjects were examined by electronystagmography for spontaneous and positional nystagmus In this way the normal limit of the speed of the slow phase was determined to be 6.5°/sec By examining 15 of the subjects twice the normal limit of the mean value of the two results was determined to be 4 5°/sec Forty nine of the subjects were calorically tested using 30° and 44°C stimulation The results were calculated as indices expressing the degree of unilateral hypofunction and directional preponderance relative to the total caloric excitability The results were evaluated for the duration of and for the maximum eye speed of the slow phase The normal limits were determined as the mean value± twice the standard deviation Our results indicate that a left side hypofunction is a normal phenomenon and that duration and maximum eye speed of the slow phase reflect different aspects of the vestibular function

The advantages of using electronystagmography (ENG) in the vestibular clinic are considerable (Aschan et al , 1956, Jongkees & Philipszoon, 1964) The method has gained world wide use during the last two decades ENG makes it possible to record nystagmus under conditions where visual inhibition of nystagmus can be excluded, and also to store and quantify the results of the vestibular examination The method is very sensitive and the examiner faces problems of the limits of normality, because it is possible to record small spontaneous and positional nystagmus beats on persons without symptoms or other signs of vestibular disturbance This necessitates that the ENG laboratory like other clinical laboratories, must examine a sample of vestibular normal subjects and by means of statistical analyses stipulate the limits of normality

The purpose of this work was to determine the normal limits of the strength (slow phase velocity) of spontaneous and positional nystagmus (SN and PN), and the normal limits of side and directional differences in the caloric test Fifty vestibular normal subjects were examined for this purpose

MATERIAL AND METHODS

The age- and sex-distribution of the 50 subjects is illustrated in Fig 1 The sex ratio was by chance exactly 1 1 The subjects were randomly picked from patients admitted to the ENT department, fulfilling following condi

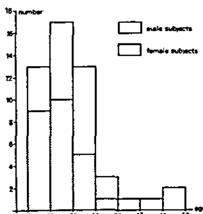
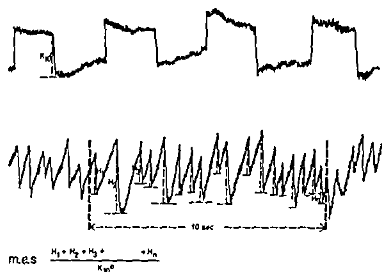


Fig 1



m.e.s $\frac{H_1 + H_2 + H_3 + \quad + H_n}{K_{10^\circ}}$

Fig 2

tions (i) age 15–50 years, (ii) no history of unconsciousness caused by head trauma, (iii) no drugs during the last 48 hours (except for oral contraceptives), (iv) no history of vertigo or dizziness, (v) normal otoscopy, and (vi) normal screening audiometry

An Elema Mingograf 34 AC amplifier with a time constant of 2 5 sec amplified the signal obtained from two electrodes placed immedia ly behind the lateral corners of the eyes ly horizontal nystagmus was recorded pontaneous nystagmus was registered with the subject in a sitting position with the eyes closed—positional nystagmus was registered from supine, left lateral right lateral and hang ing head positions The caloric stimulation was performed with a Heto Ototherm auto matic irrigation apparatus irrigating the ear canal for 30 sec with 190 ml water at 30° and 44°C The eyes were closed during the caloric test If the induced nystagmus showed any sign of suppression, the subject was asked to perform simple arithmetic calculations (e g counting backwards) Whenever it was pos sible, the subjects were re examined 24 hours after the first examination (15 subjects) The intention of this procedure was to determine normal limits for patients examined twice and thereby obtain a greater accuracy than is pos sible with the result of a single examination

The results of the examinations for SN and PN were evaluated for the eye speed of the slow phase The results of the caloric tests were evaluated for the duration of the nystagmus and the maximum eye speed of the slow phase (m e s) The latter, m e s , was calculated in two different ways First we used our rou tine method The most intense 10 sec period was chosen and the amplitudes were meas ured, added and divided with the calibration of 10° eye deviation (Fig 2) The results ob tained with this method are expressed as the mean amplitude per second during the cul mination Strictly, this speed is not the eye speed of the slow phase, the value being slightly less than the true speed of the slow phase as the time used for the quick phases during the 10 sec period should be excluded As the speed of the quick phase is relatively constant and 4–5 times as great as the speed of the slow phase, the calculated value is a usable measure of the m e s of the slow phase The method is simple to use and the results are easily reproduced The classical measuring of angles is inaccurate and necessitates more ex act calibration of the amplifier, and further more it is difficult to obtain a mean value for a reasonable period of time The other method for calculation of the eye speed was based on the output from a derivation unit coupled to

Table I *Eye speed (°/sec) of spontaneous and positional nystagmus during first examination*

No	SN	Supine	Left side	Right side	Hang head
4	+1.2	0	+5.0	−4.7	+2.5
5	+1.3	+2.0	+2.7	+3.0	0
7	−2.5	0	0	−2.5	+2.5
8	+1.3	+5.0	+4.7	+6.3	+5.0
9	0	0	+3.7	0	+2.5
10	−8.2	−6.5	−6.8	−7.6	−8.8
11	0	0	+3.1	−3.7	0
13	0	0	0	−4.0	0
14	+2.6	0	+3.8	+2.9	0
15	−4.0	0	+7.0	−2.0	0
17	0	0	0	−3.6	−1.4
18	−3.8	0	0	−1.9	0
19	0	0	+2.2	0	0
20	0	0	+2.9	0	0
22	0	0	0	−3.7	0
23	−2.5	−2.2	0	−3.1	−2.8
24	−2.8	0	0	0	0
25	0	0	−2.0	0	−3.7
26	0	−2.9	+4.7	−8.2	0
28	0	0	+4.3	0	0
29	−5.0	−2.3	+4.7	−5.3	0
30	−2.1	−2.9	0	−3.2	−4.4
35	+3.7	+2.8	+5.0	+2.5	+2.8
38	0	0	0	−4.0	0
40	0	0	+2.9	−5.0	0
44	+2.9	+3.2	+2.1	+3.8	+2.1
45	−2.0	−2.0	0	0	0
46	0	0	+4.1	0	0

the amplifier (Henriksson, 1955) This curve is dominated by peaks representing small speed variations during the slow phases It is very difficult to determine the mean value of the eye speed from this curve and it is necessary to measure several different calibration factors to obtain the true value of the eye speed The results obtained with the latter method were very similar to the results of the former method The results were excluded from this is work, as we decided to continue with the old method in the daily work of our laboratory Nevertheless, the curve obtained proved valuable for the selection of the 10 sec of culmination

The final results of the caloric tests were calculated from following formulas

Index of side difference

$$I_{sd} = \frac{(L_{44}+L_{30})-(R_{44}+R_{30})}{L_{44}+L_{30}+R_{44}+R_{30}} \times 100\%$$

Index of directional preponderance

$$I_{dp} = \frac{(L_{30}+R_{44})-(L_{44}+R_{30})}{L_{44}+L_{30}+R_{44}+R_{30}} \times 100\%$$

RESULTS

Examination for spontaneous and positional nystagmus

Twenty-eight (56%) of the 50 subjects had SN and/or PN (see Table I) Of these, 15 (30%) subjects had SN Subjects not mentioned in Table I had neither SN nor PN As is shown in the table, the values measured were between 1 2 and 8 8°/sec Technically, it is difficult to measure values below 2°/sec It is reasonable to suppose that it would be possible to measure SN and PN of values below 2°/sec in a majority of subjects if a more sensitive ENG apparatus were used The result would then be a distribution of the eye speeds of SN and PN with a high density of values from 0 to 2°/sec and a decreasing density for higher values Quite likely, the distribution of the logarithm of the eye speed would be normal Fig 3 demonstrates that this is the case, when values below 2°/sec are excluded because of their uncertainty The normal limits of a biological property are usually defined as including all values around the mean value (plus/minus twice the standard deviation) The only limit of interest in the present case is the upper limit, meaning that theoretically 97 72% of normal subjects are included in the *normal range In this way the normal limit can* be determined to be a little below 6.5°/sec This is in good agreement with results obtained with similar methods of examination (Jongkees & Philipszoon, 1964)

The signs in Table I refer to the direction of the nystagmus, where "−" denotes directed to the left, and "+" denotes directed to the right If the normal limits are forgotten, it is possible to classify the nystagmus according to Nylen (1953) Six subjects can be classified as type I (direction-changing), another 6 subjects as type II (direction fixed) and the rest as typ

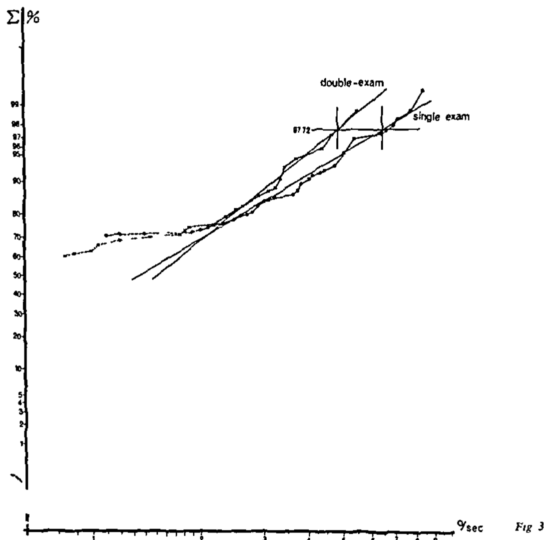


Fig 3

III (irregular, transitory) Nine subjects had PN in one position (of these, 2 also had SN), 8 subjects had PN in two positions (of these, 3 had SN), 6 subjects had PN in three positions (5 also had SN) and 4 subjects had PN in all four positions (all 4 also had SN) While it is impossible to quantify PN using Nylen's classification for statistical evaluation, the number of positions with nystagmus is a quantitative parameter There is a slight tendency for an increasing mean eye speed of the nystagmus with an increasing number of positions, about 0 2°/sec per position The correlation coefficient does not differ significantly from zero ($p>0$ 05), so that the correlation is weak It is possible that the number of pos tions should be regarded as a separate para meter, as done by Jongkees & Philipszoo (1964)

Subjects examined twice

Fifteen subjects were re examined 24 hour after the first examination The results ar listed in Table II It is obvious from the tabl that SN and PN are very variable quantities i this study Statistically, the mean of all SN an PN does not differ from the mean value of th difference from first to second examination

The normal limit of the mean value of tw examinations can be estimated in the sam

Table II *Eye speed (°/sec) of spontaneous and positional nystagmus of subject examined twice*

No	Exp no	SN	Supine	Left side	Right side	Hang head
2	1	0	0	0	0	0
2	2	0	0	0	0	0
4	1	+1.2	0	+5 0	−4 7	+5 2
4	2	0	0	0	0	0
16	1	0	0	0	0	0
16	2	0	0	0	0	0
22	1	0	0	0	−3 7	0
22	2	0	0	0	0	0
23	1	−2.5	−2 2	0	−3 1	−2 8
23	2	−4 1	−3.5	−3 5	−7 6	3.8
25	1	0	0	−2 0	0	−3 7
25	2	0	−1 7	0	0	−2 7
26	1	0	−2 9	+4 7	−8.2	0
26	2	0	−2 6	0	−5 3	0
27	1	0	0	0	0	0
27	2	0	−1 8	−2 9	+3 6	0
28	1	0	0	+4 3	0	0
28	2	0	0	0	0	0
30	1	−2 1	−2 9	0	−3 2	−4 4
30	2	0	−1 8	−2 1	−4 1	−2 4
37	1	0	0	0	0	0
37	2	0	0	0	0	0
40	1	0	0	+2 9	−5 0	0
40	2	0	0	+3 2	−3 6	0
42	1	0	0	0	0	0
42	2	0	0	0	0	0
46	1	0	0	+4 1	0	0
46	2	0	+2 5	+5 0	0	+2 1
49	1	0	0	0	0	0
49	2	0	0	0	0	0

way as done above (see Fig 3) In this way the normal limit is determined to be a little above 4 5°/sec

SN and PN in relation to age and sex

The mean age of subjects presenting SN and/or PN is identical with the mean age of subjects without SN and/or PN 24 2 years In the group with SN and/or PN, 64% are men, whereas in the group of subjects without SN or PN, only 32% are men A χ^2 test shows that this difference is significant ($p<0\ 05$) Furthermore, 40% of the women with SN and/or PN used oral contraceptives and 27% of the women without SN or PN did so though this difference is not significant ($p>0\ 05$)

Results of the caloric tests

The indices of side difference (I_{sd}) and of directional preponderance (I_{dp}) are the final results of the caloric tests From the formulas it can be shown that a positive value of I_{sd} indicates a right side hypofunction and a negative value a left side hypofunction Correspondingly, a positive value of I_{dp} indicates a right-directional preponderance and a negative value a left-directional preponderance The results are listed in Table III Fig 4 demonstrates that the functions are normally distributed One of the mean values, I_{sd} of duration, differs significantly from zero

Subjects examined twice

Mean values and standard deviations of the mean values of the results of the double examinations are listed in Table III Only two of

Table III *Values for side difference I_{sd} and directional preponderance I_{dp} (°/sec)*

Parameter	Mean value	S D	Normal limits x−2 S D	x+2 S D
Single examination (all subjects)				
I_{sd} duration	−5 4	6 7*	−18 8	+ 8 0
I_{sd} m e s	+0 2	9 4	−18 6	+19 0
I_{dp} duration	−3 8	17 8	−39 4	+31.8
I_{dp} m e s	+2 4	12 0**	21 6	+26 4
Double examination (mean value)				
I_{sd} duration	−5 9	4 8*	15 5	+ 3 7
I_{sd} m e s	−3 3	7 2	−17 7	+11 1
I_{dp} duration	−7 4	13.2	−33 8	+19 0
I_{dp} m e s	+2 0	7 0**	−12 0	+16 0
Subjects with SN/PN (first examination)				
I_{sd} duration	−5 0	7.2	−19 4	+ 9 4
I_{sd} m e s	+1 4	10.3	−19.2	+22 0
I_{dp} duration	−6 4	21 6***	−49 6	+36 8
I_{dp} m e s	+0.3	13 4	−26.5	+27 1
Subjects without SN/PN (first examination)				
I_{sd} duration	−6 0	6 0	−18 0	+ 6 0
I_{sd} m e s	−1.2	8.2	−17 6	+15.2
I_{dp} duration	−2 4	10 8***	−24 0	+19 2
I_{dp} m e s	+5 1	9 6	−14 1	+24.3

Significant differences *=$p<0\ 05$ **=$p<0\ 01$ ***=$p<0\ 01$

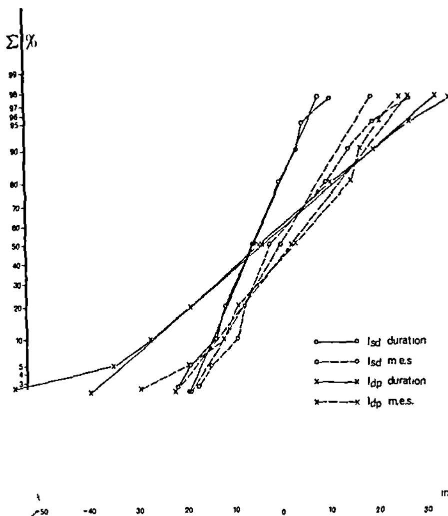


Fig 4

the standard deviations of the mean values of double examinations are significantly smaller than the standard deviations of the corresponding single examinations There is no significant difference between the mean values of double and single examinations

SN and PN compared with the results of the caloric tests

Mean values and standard deviations of each index were calculated for subjects with and without SN and/or PN The results are shown in Table III The only significant difference was found for the standard deviations of I_{dp} of duration The other differences were not significant The presence of SN and/or PN explains the large standard deviation of the original function I_{dp} of duration

DISCUSSION

The procedure in our ENG examination is very much like the procedure described by Jongkees & Philipszoon (1964) The frequency of SN and PN in a normal sample depends on the sensitivity of the ENG apparatus used Consequently, it is difficult to compare the frequencies of SN and PN for different authors' studies Jongkees & Philipszoon used two different limits in their discussion, 6 and 7°/sec The latter value is said to be the lower limit of visually observed nystagmus through Frenzel's glasses This must be a very uncertain limit, as it is not the slow phase of the nystagmus that is observed through Frenzel's glasses, but the amplitude, which might be completely independent of the slow phase (Tibbling & Henriksson, 1968, Pfaltz, 1974)

The only safe way to determine the limits of normality is the statistical way, which analyses the data for a sample of normal subjects —as in all other clinical tests By adopting this method we have determined the limits of the eye speed of the slow phase of spontaneous and positional nystagmus to be 6 5°/sec Another way to describe PN is by the number of positions in which the PN is present Our results do not show a good enough correlation between the two parameters It is possible that the number of positions must be regarded as a separate parameter This complicates the evaluation of the SN and PN examination

It is clinical practice to re examine a patient when there is doubt about the interpretation of the results of a clinical test For statistical reasons it is necessary to use the mean value of two test results and to compare this mean value with the normal limits of a double tested normal sample The normal limit of our double tested sample is, as expected lower than the normal limit of the single tested

The occurrence of SN and PN appeared more frequently in men than in women but we are unable to explain this Oral contraceptives did not significantly influence the frequency of SN and PN

The result of the caloric test can be expressed in indices or in absolute values The advantage of the indices is that the values are directly related to the two diagnoses possible unilateral hypofunction and directional preponderance and that these values are relative to the total caloric excitability Left side hypofunction in normal subjects has been shown by other authors (Molnar & Torok, 1974) who demonstrated that it is not a result of a fixed sequence of irrigation The presence of SN and/or PN only influences the standard deviation of the I_{dp} of duration, not the m e s This demonstrates that SN and PN prolong the duration but do not affect the strength of the nystagmus indicating that the two parameters duration and m e s reflect different aspects of the vestibular function (Henriksson, 1956)

Normal limits of double examinations were evaluated on the basis of the mean values of the two examinations Mean values of these results did not differ significantly from the mean values of single examinations All standard deviations were, as expected, less (only two of them significantly)than the standard deviations of single examinations

ZUSAMMENFASSUNG

Funfzig normale Personen wurden mittels Elektronystagmographie für Spontan und Positionsnystagmus untersucht In dieser Weise wurde die Normalgrenze der Geschwindigkeit der langsamen Phase bestimmt (6.5°/sec) Funfzehn Personen wurden zweimal untersucht dabei wurde es möglich die Normalgrenze des Durchschnittes der zwei Untersuchungen zu bestimmen (4,5°/sec) Neunundvierzig der Normalpersonen wurden mit 30 und 44° heißem Wasser kalorisch gepruft. Die Resultate wurden in Indizes die die relative einseitige Hypofunktion und das Richtungsubergewicht ausdrucken ausgerechnet Die Resultate wurden für sowohl Duration wie maximale Augengeschwindigkeit der langsamen Phase (m e s) gewurdigt Die Normalgrenzen wurden als Durchschnitt± zweimal der Standarddeviation bestimmt Unsere Resultate zeigen daß eine linksseitige Hypofunktion eine normale Erscheinung ist und daß Duration und m e s verschiedene Seiten der Vestibularfunktion widerspiegeln

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RESPONSE OF SINGLE PURKINJE NEURONS IN THE FLOCCULUS OF ALBINO RABBITS TO CALORIC STIMULATION

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Abstract The response of Purkinje neurons of the flocculus to caloric stimulation was investigated in the Urethane–chlorarose anesthetized rabbit Twenty five of 37 flocculus neurons which responded to ipsilateral caloric stimulation showed an increase in firing response while 12 neurons showed a decrease Fifteen of 28 flocculus neurons which responded to contralateral caloric stimulation showed an increase while the firing of 13 neurons was decreased Forty-one percent of flocculus neurons responded to ipsilateral caloric stimulation and 41 % responded to caloric stimulation of both sides Eighteen percent of flocculus neurons responded only to contralateral stimulation The ipsilateral flocculus may thus be ...onsible for the major control of the primary vestib-... flow in the cerebellum

The processing of the bilateral input into the vestibular system remains the subject of an increasing number of the studies The role of the contralateral labyrinth in the processing of vestibular input has previously been documented (Matsuoka, 1969, MacCabe et al, 1969, Matsuoka et al, 1971, Shimazu & Precht, 1966, Shinoda & Yoshida, 1975) Shimazu & Precht (1966) described two types of vestibular neurons in the cat (type I and type II) which responded to electrical stimulation and rotation In type I there was an excitatory input from the ipsilateral side, while the input from the labyrinth of the other side was inhibitory Type II was the converse Using caloric stimulation, Matsuoka (1969) demonstrated similar findings in the lateral vestibul[ar] nucleus of the cat However, he reported a[d]ditional types of response including a revers[al] of the type I and type II found by Shimazu & Precht (1966) It is conceivable that these re[sults] indicate an antagonistic principle in th[e] central nervous system to stimulation

In studies on the role of the flocculus in th[e] vestibulo oculomotor system (Ito, 1972, Ito e[t] al, 1973*a*, *b*, Ghelarducci et al, 1975) Ghelarducci reported two major types of neuron (inphase and outphase) in the flocculus of a[n] alert rabbit which was rotated sinusoidally o[n] a turntable during visual stimulation These two types of neurons corresponded to type [I] and type II at the vestibular nucleus level i[n] the cat

In this rotating experiment, both sides of the labyrinth are stimulated simultaneously, an[d] therefore, unilateral participation of phase generation cannot be discerned

According to the results of Shinoda & Yoshida (1975), the flocculus also receives a[n] input from the contralateral vestibular orga[n] via the contralateral vestibular nucleus and cerebellum Generally speaking, only the shortest pathway could be observed with electrical stimulation Natural stimulation is therefore a more acceptable method for studying overall responses, although quantitative analysis of the data presents difficulties We have applied caloric stimulation to both

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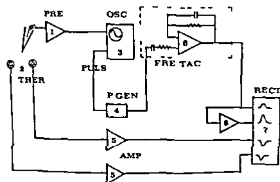


Fig 1 Recording system 1 PRE Preamplifier with high impedance input 2 THER Thermistor to monitor temperature 3 OSC Oscilloscope (Nihon Koden VC 7) to monitor the sliced spike 4 P GEN Pulse comes from triggered output of oscilloscope 5 AMP Usual amplifier to amplify thermistor record 6 FRE TAC Frequency tachometer to transform pulse into analog output has Norton type operational amplifier (LM 3900) R C was chosen to eliminate the small ripples in the responses 7 RECT 4-channel recticorder (Sanei Sokki Model 114) 8 AMP

labyrinth organ successively and examined the commissural effect of contralateral caloric stimulation of neurons in the flocculus

MATERIALS AND METHODS

Thirteen albino rabbits weighing 2–3 kg were anesthetized with Urethane (700 mg/kg i v) and Chlorarose (30 mg/kg i v) During the experiment, Urethane was given as required After tracheotomy, artificial respiration was maintained and the animal was paralysed with Gallamine (10 mg/kg i v) and fixed in a stereo taxic instrument After excising the external ear, both sides of the ear drum and tympanic cavity of the animal were exposed A metal thermistor and small tube of 0 5 cm in diameter were inserted into the tympanic cavity near the horizontal semicircular canal The tube was used to pour cold and hot water (2 ml of 10°C or 45°C) into the tympanic cavity and the thermistor in the tympanic cavity monitored temperature changes at the semicircular canal site A hole of about 1 0 cm in diameter was drilled in the bone lying over the paraflocculus in order to insert a microelectrode into the flocculus After trepanation of the dura, a glass microelectrode (2 0 to 10 0 M) was inserted and the neuronal activity was recorded at the cortical level of the flocculus (7 0 to 11 0 mm from the surface) Caloric stimulation was applied to one side and responses recorded The opposite side was then stimulated and recordings again made The Purkinje neuron was identified by the presence of complex spikes (Thach, 1968) For analysis of the data, single neuronal activity was transformed into a pulse with the aid of a slicer device, then converted into an analog output using a frequency tachometer Thus, firing frequency of a neuron could be calculated by this analog output Slice level was always monitored by an oscilloscope (Fig 1) All data were registered on a 4-channel paper recticorder (Sanei Sokki, Model 114) An electrolytic lesion was made in the flocculus by replacing the glass electrode with a metal one and applying current This lesion was examined to confirm the location of electrode placement

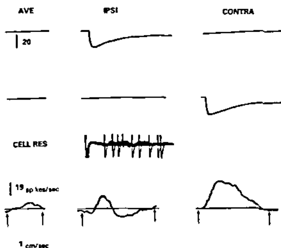


Fig 2 Caloric stimulation and neuron responses The top trace shows record of temperature changes with ipsilateral caloric stimulation and the middle trace those with contralateral stimulation The vertical line shows 20°C/cm The bottom trace shows neuronal responses to caloric stimulation Arrow indicates the time when measurement was done Simple spikes are shown in the middle

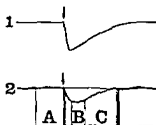


Fig 3 Schema of calculation 1 Thermal changes in the middle ear to caloric stimulation 2 Neuronal response to caloric stimulation Arrow indicates beginning of response *A* Prestimulus level (usually 30 sec) *B* Peak response (usually 10 sec–20 sec) *C* Total response (usually 60 sec)

RESULT

Seventy-seven flocculus neurons with complex and simple spikes were observed The neuronal activity following caloric stimulation is shown in Fig 2 After irrigation with cold water, the thermal change in the tympanic cavity continued for 60 to 100 sec

Areas of response designated A, B and C (Fig 3) were measured using a planimeter Area A corresponds to the firing frequency before stimulation, area B to the maximum k or valley response during stimulation and area C to the entire period of response following stimulation

The Purkinje cell fired spontaneously *sometimes in bursts, so that an area which wa* stable for 30 sec before stimulation served a area A Area C was measured over the whol period following stimulation during which modulation was recognized An average modulation response could usually be observed fo 120 sec Some neurons showed a large modulation down to 3% of normal, while the duration of the response continued to 150 sec o longer Probably, these neurons were directl connected to primary vestibular fibres Are B was chosen as that corresponding to a perio of 10–20 sec when the largest or deepest valley of each response occurred The latency between the onset of stimulation and the peak o valley of the response varied, case by case from 10 to 30 sec

The ratio of the area C/area A represente a normalized response and the ratio of the are B/area A represented a normalized peak response A slight change in firing frequenc was observed in recording of the respons (Fig 2) However, it was possible to deter

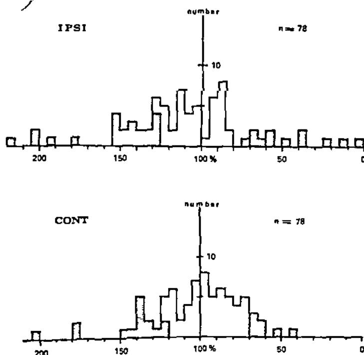


Fig 4 Distribution of peak response to ipsilateral and contralateral stimulation White Non modulated neurons Black dots Modulated neurons

Table I *Modulated neuron during caloric stimulation*

Response type	Ipsilateral stimulation	Contralateral stimulation
Increased type	25 (32%)	15 (19%)
Decreased type	12 (16%)	13 (17%)
No change	40 (52%)	49 (64%)

mine whether or not a neuron was modulated by defining a neuron modulated by caloric stimulation as having over 125% of normalized peak response or valley of less than 75%.

The peak response of all neurons to caloric stimulation is shown in Fig 4. The ordinate is the number of neurons with each block representing a single flocculus neuron. The abscissa represents normalized peak percentage. The dotted block shows neurons which showed exceptional modulation. The range of response to ipsilateral stimulation was wider than that to contralateral stimulation. The value of contralateral modulation was not as high as seen in the case of ipsilateral stimulation. The total number and the percentage on one side is shown in Table I. The maximal amount of decrease in response to ipsilateral caloric stimulation was down to 3% of normal at the valley. With contralateral caloric stimulation, moderate modulation was seen in all cases. The typical response patterns of the flocculus neurons responding to bilateral caloric stimulation are shown in Fig 5. The responses were divided into three groups as shown in Table II. The first group was those neurons which responded only to ipsilateral caloric stimulation (19 neurons, 41%) and the second, those neurons which responded to both ipsilateral and contralateral stimulation (19 neurons, 41%). In the second group, types C and E, which correspond to types I and II in the brain stem (Shimazu & Precht, 1966), included 8 neurons (18%) and 4 neurons (9%) respectively. Responses of the second group were however, moderate compared with those seen only to ipsilateral stimulation. The third group of neurons responding only to contralateral caloric stimulation included 8 neurons (18%).

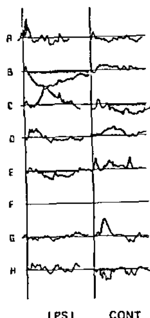


Fig 5. Typical response to consecutive bilateral stimulation.

DISCUSSION

The vestibular input to the flocculus has been demonstrated anatomically (Brodal & Torvik, 1957, Brodal & Høivik, 1964) and electrophysiologically (Llinas et al., 1971, Wilson et al., 1974, Shinoda & Yoshida, 1975). The latter authors described cells responding to contralateral electrical stimulation with a one

Table II *Number of the neurons modulated by consecutive bilateral caloric stimulation*

Type	IPSI	CONT	NUM	
1 A	↑	–	11	19 41%
1 B	↓	–	8	
2 C	↑	↓	8	19 41%
2 D	↑	↑	7	
2 E	↓	↑	4	
2 F	↓	↓	0	
3 G	–	↑	3	8 18%
3 H	–	↓	5	

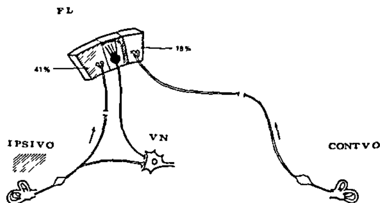


Fig 6 Schematic illustration of the inner vation from both labyrinths

synapse delay and a two synapse delay In their work, the impulse from the contralateral side was conveyed via contralateral medial vestibular nucleus and cerebellum As the flocculus has input of second order fibres from the vestibular nucleus as reported by Brodal & Torvik (1957), the inputs from the contralateral labyrinth are assumed to be conveyed via these pathways

Shinoda & Yoshida (1975) stated that 35% of neurons activated by electrical stimulation of the ipsilateral labyrinth increased their fir- frequency with ipsilateral acceleration and ed it with contralateral acceleration In ition, 35% of neurons activated by electrical stimulation of the contralateral labyrinth decreased their firing frequency in response to ipsilateral acceleration and decreased it during contralateral acceleration In our experiment, the former figure corresponded to 30 7% (Type C of the cells which had an increased response to ipsilateral caloric stimulation) and the latter to 28 5% (Type E of the cells which had an increased response to contralateral caloric stimulation) Wilson et al (1974) reported that 39% of neurons in the vestibulocerebellum activated by ampullary nerve stimulation had bilateral inputs This value is in good agreement with the 41% of neurons in our data which responded to bilateral caloric stimulation as shown in Fig 6 Therefore, the values described here suggest a constant ratio of each vestibular contribution to the flocculus regardless of species differences

The response of the Purkinje neurons in t cerebellum of cats to caloric stimulation w reported by Ferin et al (1972) He describ neurons which responded to caloric stimu tion in the vestibulo cerebellum, and the r sponse of the simple spike showed its peak about 10 sec after stimulation, and ended wi in 50 sec In the present experiment, the du tion of the caloric response was also short that the response usually ceased within 60 se but in the case of two neurons, the respon was as long as 150 sec or more These seem be the typical pattern of Purkinje neuron r sponse to caloric stimulation in the vestibul cerebellum

When using caloric stimulation, Matsuol (1969) found similar results in the vestibul nucleus in the cat He classified the respons into five types and all five were found in th present experiment However, the percen ages of neurons responding to caloric stimul tion of both sides were higher than that in th flocculus The vestibular neurons usual showed a time course of 150 sec or mor Therefore, it would appear that the majority neurons in the flocculus are not directly co nected to the canal It must be pointed ou however, that the temperature of the water i caloric stimulation was cooler (10°C) than th used by Matsuoka and the flocculus has man inputs via the brain stem and other periphe systems (Maekawa & Simpson, 1973) In addi tion, there were silent cells which did no respond to caloric stimulation

Types I and II in the vestibular nucleus (Shimazu & Precht, 1966) correspond to types C and E in Fig 5, although their frequency was less than that expected in the vestibular nucleus as shown in the data of Matsuoka (1969)

In the experiment of Ghelarducci et al (1975), spikes were recorded from the flocculus of an alert rabbit using sinusoidal rotation stimulation Neurons showed various responses, almost all of which could be fitted into a sine curve combined with phase shift (−180° to +180°) and amplitude change In polar expression, these responses spread from outphase type (−180°±45°) and inphase type (±45°) to intermediate type (remaining phase) The inphase and outphase type correspond to the increase and decrease type in our experiment The increase or decrease response was observed just after the application of the caloric stimulation to one side of the labyrinth but there was no clear relationship to these responses from the other side of the labyrinth Therefore, we assume that both labyrinths participate in these phase generations

The ratios of the contribution from the ipsilateral bilateral and contralateral labyrinth to the flocculus were 41 %, 41 % and 18 %, respectively and the ipsilateral flocculus may, therefore be responsible for the major control of the primary vestibular signal flow into the cerebellum (Fig 6)

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ZUSAMMENFASSUNG

Die Antwort von Purkinje Zellen des Flocculus auf kalorische Stimulation wurde beim Urethan-Chloralose anästhesierten Kaninchen untersucht 25 von 37 Flocculusneuronen die auf ipsilaterale kalorische Stimulation reagierten zeigten eine Zunahme 12 Neurone zeigten eine Abnahme der Entladungstätigkeit 15 von 28 Neuronen die auf kontralaterale kalorische Stimulation reagierten zeigten eine Zunahme 13 eine Abnahme 41 % der Flocculusneurone antworteten auf ipsilaterale kalorische Stimulation und 41 % der Zellen antworteten auf kalorische Stimulation beider Seiten 18 % reagierten nur auf kontralaterale Stimulation Der ipsilaterale Flocculus konnte deshalb wesentlich für die Kontrolle des primären vestibularen Einganges ins Kleinhirn verantwortlich sein

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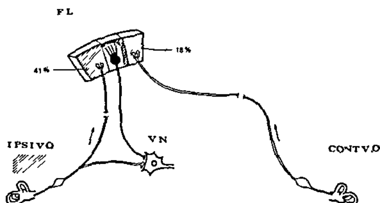


Fig 6 Schematic illustration of the inner vation from both labyrinths

synapse delay and a two synapse delay In their work, the impulse from the contralateral side was conveyed via contralateral medial vestibular nucleus and cerebellum As the flocculus has input of second order fibres from the vestibular nucleus as reported by Brodal & Torvik (1957), the inputs from the contralateral labyrinth are assumed to be conveyed via these pathways

Shinoda & Yoshida (1975) stated that 35% of neurons activated by electrical stimulation of the ipsilateral labyrinth increased their fir- frequency with ipsilateral acceleration and ased it with contralateral acceleration In 35% of neurons activated by electrical stimulation of the contralateral labyrinth decreased their firing frequency in response to ipsilateral acceleration and decreased it during contralateral acceleration In our experiment, the former figure corresponded to 30 7% (Type C of the cells which had an increased response to ipsilateral caloric stimulation) and the latter to 28 5% (Type E of the cells which had an increased response to contralateral caloric stimulation) Wilson et al (1974) reported that 39% of neurons in the vestibulo cerebellum activated by ampullary nerve stimulation had bilateral inputs This value is in good agreement with the 41% of neurons in our data which responded to bilateral caloric stimulation as shown in Fig 6 Therefore, the values described here suggest a constant ratio of each vestibular contribution to the flocculus regardless of species differences

The response of the Purkinje neurons in t cerebellum of cats to caloric stimulation w reported by Ferin et al (1972) He describ neurons which responded to caloric stimul tion in the vestibulo-cerebellum, and the r sponse of the simple spike showed its peak about 10 sec after stimulation, and ended wit in 50 sec In the present experiment, the dur tion of the caloric response was also short that the response usually ceased within 60 se but in the case of two neurons, the respon was as long as 150 sec or more These seem be the typical pattern of Purkinje neuron r sponse to caloric stimulation in the vestibul cerebellum

When using caloric stimulation, Matsuok (1969) found similar results in the vestibul nucleus in the cat He classified the response into five types and all five were found in th present experiment However, the percent ages of neurons responding to caloric stimul tion of both sides were higher than that in th flocculus The vestibular neurons usuall showed a time course of 150 sec or more Therefore, it would appear that the majority o neurons in the flocculus are not directly con nected to the canal It must be pointed out however, that the temperature of the water in caloric stimulation was cooler (10°C) than that used by Matsuoka and the flocculus has many inputs via the brain stem and other peripheral systems (Maekawa & Simpson, 1973) In addi tion, there were silent cells which did not respond to caloric stimulation

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ACOUSTIC MEASURES FOR DETECTING LARYNGEAL PATHOLOGY

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Abstract Perturbations in the fundamental pitch and in the peak amplitude of the acoustic signal derived with a contact microphone system were investigated for the purpose of developing useful measures for the detection of laryngeal pathology Sixty three patients with various laryngeal pathologies and 31 normal subjects were studied In 14 cases high speed motion pictures of the larynx were taken in order to examine the physiological mechanisms giving rise to the perturbations in the acoustic signal The collected acoustic data were statistically processed and a critical ellipse was computed for each subject category The significance of the concept of a "normal standard" was discussed It was pointed out that the physiological mechanisms for the acoustic perturbations were rather complicated and that many physiological aspects may have to be taken into account The need for basic physiological research in relation to pathologic speech production was emphasized

It has long been known that patients with laryngeal diseases often develop a change in voice quality which may be referred to as "hoarseness" Since such a vocal alteration can reveal itself at a very initial stage of the pathology, many attempts have been made to utilize vocal cues for the purpose of an early detection of certain laryngeal lesions On the other hand, it has been generally accepted that some types of laryngeal pathologies are associated with certain vocal characteristics, and that trained laryngologists are therefore often able to predict the laryngeal status simply by listening to the patient's speech, if the pathology is at a certain stage of development

These two feasibilities, i e , early detection and differential diagnosis, have obviously motivated and enhanced the study of speech produced by patients with laryngeal disorders A great amount of research on pathologic voices has thus been conducted mainly on the basis of auditory perception Most of these studies, however, have been more or less subjective, and have accordingly resulted in considerable confusion of concepts and terms It is apparent that auditory perception of hoarseness can be influenced by many factors, perhaps even by many extralaryngeal elements

Some acoustic studies, such as spectral analysis of hoarse voices, have also been attempted in the past It has not been an easy task to relate the results of such analysis to the underlying pathology in the larynx, however, since the effects of the supraglottal structure could not be readily accounted for, and since the results of such analyses revealed a wide range of variability It is only during the last one and a half decades that investigators have started to present precise and quantitative acoustic data on pathologic speech This may be due to the recent advancement in the digital means of processing and to an improved understanding of the effect of the supraglottal structure upon speech

This study was supported by the U S P H S grant (no NS 08177)

Table I *Laryngeal pathology, age and sex of the patients subjected to the acoustic study*

Subject Number	Pathology	Age	Sex	Subject Number	Pathology	Age	Sex
Group 1 Neoplasms				*Group 3 Paralysis*			
H 1	Laryngeal cancer	50	m	U 9	l unilateral paralysis	16	m
A 1	Laryngeal cancer	55	m	R-4	l unilateral paralysis	44	m
A 2	Laryngeal cancer	61	f	R 5	l unilateral paralysis	62	m
A-4	Laryngeal cancer	57	m	U 37	l unilateral paralysis	28	f
A 5	Laryngeal cancer	65	m	O 1	l unilateral paralysis	53	f
A 11	Laryngeal cancer	69	m	D 2	r-unilateral paralysis	55	m
O 2	Benign tumor	61	f	U 29	r unilateral paralysis	53	f
U 24	Benign tumor	77	m	R 3	Bilateral paralysis	60	m
U 1	Vocal nodule	33	f	U 28	Bilateral paralysis	37	m
U 5	Vocal nodule	11	m				
D 1	Vocal nodule	40	m				
U 16	Vocal nodule	25	f	*Group 4 Other pathologies*			
U 25	Vocal nodule	63	f	U 11	Hemi laryngectomized	56	m
U 27	Vocal nodule	26	m	U 23	Hemi laryngectomized	45	m
U 36	Vocal nodule	48	f	U 17	Partial laryngectomized	76	m
U 3	Vocal polyp	50	f	U 10	Epiglottis resected	60	f
U 7	Vocal polyp	44	f	U 32	Laryngeal trauma	60	f
U 22	Vocal polyp	39	m	U 20	Polyp removed	72	f
R 2	Contact granuloma	69	m	U 21	Polyp removed	50	f
				U 35	Polyp removed	51	f
Group 2 Inflammation				U 14	Sulcus vocalis	66	m
U-4	Chronic laryngitis	48	f	U 13	Mutational disturbance	20	m
U-8	Chronic laryngitis	29	f	U 19	Spastic dysphonia	39	f
O 3	Chronic laryngitis	48	m	U 12	Functional dysphonia	41	f
U 15	Chronic laryngitis	44	m	U 2	Functional dysphonia	50	f
U 30	Chronic laryngitis	62	m	U 33	Functional dysphonia	43	f
U 31	Chronic laryngitis	8	f				
U 34	Chronic laryngitis	75	f				

Among these recent studies, those by Lieberman (1961, 1963) have drawn the strong attention of many researchers He reported that a ' pitch perturbation factor might be of use in the detection of laryngeal pathology Subsequent researchers (Smith & Lieberman, 1964, Moore & Thompson, 1965, Crystal & Jackson, 1970, Hecker & Kreul, 1971, Koike, 1973) also showed that perturbations in the fundamental period of speech are sensitive to some pathologies in the larynx By applying a technique of time series analysis, Koike (1969) demonstrated that the behavior of the peak amplitude of vowels also bears information attributable to certain laryngeal pathologies

Although these studies have indicated considerable promise for the development of some acoustic indices which may be useful for both the early detection and the differential diagnosis of laryngeal pathologies not much thought has been given to the essential significance of the norms for such indices A study which considers the meaning of such norms themselves, therefore, seemed to be justifiable

Also, it is a fact that basic physiological mechanisms which account for such acoustic phenomena as mentioned above have not yet been sufficiently explicated There is little empirical data, for example, on how and why such events as the pitch or amplitude perturbations occur in reference to laryngeal vibration, even though some speculations have been given in the past The need for physiological data which clarify the basic mechanisms causing such acoustic events does not require lengthy explanation

It should be mentioned here that there may be many other acoustic parameters relevant to pathologic conditions in the larynx than the pitch and amplitude perturbations which are discussed in the present article The behavior of the inverse residue pulse (Koike & Mar

Table II *Laryngeal status, age and sex of the subjects on whom high speed motion pictures of the larynx were taken*

Subject Number	Pathology	Age	Sex
K 3	Laryngeal cancer	39	m
K 5	Laryngeal cancer	66	f
K 15	Laryngeal cancer	40	m
K 9	Benign tumor	70	m
K 16	Leucoplacia	46	f
K 2	Vocal polyp	58	m
K-4	Vocal polyp	42	m
K 10	Vocal polyp	62	m
K 12	Vocal polyp	59	m
K-6	l-unilateral paralysis	36	f
K 14	l unilateral paralysis	30	f
K 17	l-unilateral paralysis	31	m
K 7	Chronic laryngitis	16	f
K 11	Sulcus vocalis	32	m
K 1	Normal	33	f
K-8	Normal	29	m
K 13	Normal	27	m

kel, 1975), for example, may be one such potentially useful index If in the future more effective acoustic parameters could be combined together, the efficiency of such indices for detecting laryngeal pathologies would become significantly higher

METHOD

Subjects

The subjects of the present study consisted of 63 patients with various laryngeal pathologies and 31 normal adults for control purposes Acoustic speech recording and indirect laryngoscopy was performed on 49 patients A detailed description of the laryngeal status and a brief summary of history were also made The pathology, age and sex of these patients are summarized in Table I The same speech recording was made for 31 normal healthy adults including 10 female and 21 male subjects who were drawn from the staff of the laboratory

Fourteen additional patients with various laryngeal diseases and 3 normal adults were subjected to study with high speed motion pictures of the larynx, along with a simultaneous recording of the acoustic speech waveform The contact microphone signal was also recorded in 7 of these cases The laryngeal status, age and sex of the photographed subjects are listed in Table II The photographed data was employed to assist the physiological interpretation of the acoustic data

Collection and analysis of acoustic data

The acoustic speech signal obtained with a conventional microphone (Electrovoice 666) was recorded on one channel of a 2-channel magnetic tape recorder (Ampex 600) The vibration of the skin in front of the trachea was also picked up by a specially constructed contact microphone (B & K ¼ inch condenser microphone no 4136 with a short rubber tube), and was registered on the other channel of the recorder

The subjects were instructed to sustain the vowel /a/ for approximately 4 sec at their comfortable pitch Then they were asked to produce the same vowel at a higher pitch, and then at a pitch lower than the comfortable level The vowels /u/, /o/, /e/ and /i/ were also recorded at their comfortable frequency Although in the present study the samples of the vowel /a/ recorded at the comfortable pitch were the main ones to be analysed, the effects of pitch levels and of different vowels were also studied on a limited subset of the subjects

It was confirmed from the high speed films that the fundamental periodicity of the contact microphone signal coincides precisely with that of the speech signals, though the phase relations between the two signals varied considerably from subject to subject, probably due to the variable locations of the contact microphone A detailed discussion on this finding was presented elsewhere (Koike & Takahashi, 1971/1972) The recorded signals were then reproduced, digitized and stored on a disk (Telefile 114) controlled by a computer (PDP-11) at a sampling rate of 20000 samples/sec (A DEC tape controlled by a PDP-8 computer was used as the storage medium instead of the Telefile disk during the initial period of the present study)

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H 1	Laryngeal cancer	50	m
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U 37	l unilateral paralysis	28	f
O 1	l-unilateral paralysis	53	f
D-2	r unilateral paralysis	55	m
U 29	r unilateral paralysis	53	f
R 3	Bilateral paralysis	60	m
U 28	Bilateral paralysis	37	m
Group 4 Other pathologies			
U 11	Hemi laryngectomized	56	m
U 23	Hemi laryngectomized	45	m
U 17	Partial laryngectomized	76	m
U 10	Epiglottis resected	60	f
U 32	Laryngeal trauma	60	f
U 20	Polyp removed	72	f
U 21	Polyp removed	50	f
U 35	Polyp removed	51	f
U 14	Sulcus vocalis	66	m
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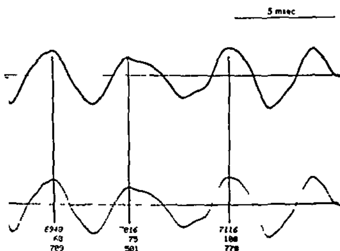


Fig 1 A contact microphone signal (*upper trace*) demonstrating an abrupt change in the period length and in the peak amplitude at the same time Lower trace denotes a dot mode representation of the same signal showing the time resolution of the analysis system Vertical lines indicate the position of the peaks automatically searched for The speaker is a patient with uni lateral recurrent nerve paralysis

me The method of defining the trend may be nproved in further studies, however, since iany other techniques are available in the rea of time series analysis

A similar long-term trend was considered lso for the series of the peak amplitude data : is apparent that the amplitude series also emonstrates various slow and smooth trends here is a possibility, however, that the same atho-physiological event causing a rapid ariation of pitch on the one hand is producing n abrupt alteration of the amplitude on the ther hand, as is seen in Fig 1 It might be nore meaningful, then, to see for some pheno nena which are peculiar to the amplitude data, f the amplitude information is to be treated as n independent acoustic parameter Further- nore, the previous report (Koike, 1969) indi- ates that a rather prolonged modulation of the mplitude bears some relevant information These considerations led to the adoption of a arger time constant for the trend line for the eak amplitude series An 11-point moving verage was thus employed to obtain the trend ine for the amplitude from the peak values The perturbations of pitch and amplitude were hen computed on the basis of the above stablished trend lines That is, the perturba ions were defined to be the absolute distances rom the smooth trend lines, both for pitch and for amplitude

A second point of consideration was as sociated with various phonatory conditions such as the overall fundamental frequency, intensity, and the articulatory environments, which were presumed to influence the short term variability of the period and amplitude measures *Inter alia*, the overall pitch level is known to affect the variability of the period considerably (Lieberman, 1961) An almost linear relationship was shown to exist between the average period length and the extent of period perturbations under certain conditions (Koike, 1973) If the period variabilities of some utterances which have different pitch levels are to be compared with each other, it would become necessary to normalize the variability in terms of the average pitch Otherwise, the magnitude of the perturbation measure may be attributable, at least in part, to the average pitch The absolute pitch per- turbation from the trend line mentioned above was therefore normalized against the average pitch The absolute amplitude perturbation also was normalized by the mean amplitude values so that any amplification of the original signal may not affect the perturbation meas- ure Since a possible effect of intensity of phonation upon the pitch and amplitude per- turbations was not explicit, though it was likely that such an effect existed, it was thought justifiable to account for such an

effect statistically together with other probably physiological variabilities

The question of articulatory environments required a serious consideration It is a fact that there exist interactions between the vocal tract configuration and the glottal source actions It is probable, therefore, that certain pathologic behaviors reveal themselves during dynamic vocal tract movements due to this interaction, and it might be feasible to take advantage of such behavior to detect some pathologic features if such an interaction could be accounted for Unfortunately, however, this interaction has not yet been theoretically explicated, and empirical data are also quite meager It was thought reasonable, therefore, to limit the object of measurement in the present study to sustained vowel utterances where the interaction between the vocal tract and the glottal behavior may safely be presumed to be steady

A *frequency perturbation quotient* (FPQ) was thus defined on the basis of above mentioned considerations

$$Q=\frac{\frac{1}{n-2}\sum_{i=2}^{n-1}\left|\frac{F_{i-1}+F_i+F_{i+1}}{3}-F_i\right|}{\frac{1}{n}\sum_{i=1}^{n}F_i} \quad (1)$$

where $F_i=1/P_i$, P_i is the duration of ith period and n is the number of periods The numerator shows the average absolute perturbation, and the denominator indicates the average fundamental frequency Similarly, an *amplitude perturbation quotient* (APQ) was defined

$$APQ=\frac{\frac{1}{n-10}\sum_{i=6}^{n-5}\left|\frac{A_{i-5}+A_{i-4}+\cdots+A_i+\cdots+A_{i+5}}{11}-A_i\right|}{\frac{1}{n}\sum_{i=1}^{n}A_i} \quad (2)$$

where A_i is the peak to peak amplitude of the ith period and n is the number of periods

The numerator expresses the average absolute perturbation of the amplitude from the trend line, and the denominator the average amplitude

Based on these quotient values a *critical ellipse* defined in general terms as follows was computed for each category of subjects If we have N sets of data, each of which has k parameter values such as

$$x_1(x_{11},x_{12},\ldots,x_{1\alpha},\ldots x_{1\beta},\ldots x_{1k}),$$
$$x_2(x_{21},x_{22},\ldots,x_{2\alpha},\ldots x_{2\beta},\ldots x_{2k}),$$
$$\vdots$$
$$x_N(x_{N1},x_{N2},\ldots,x_{N\alpha},\ldots x_{N\beta},\ldots x_{Nk})$$

$$\text{Average}\quad(\bar{x}_1,\bar{x}_2,\ldots,\bar{x}_\alpha,\ldots\bar{x}_\beta,\ldots\bar{x}_k)$$

for a given homogeneous category such as laryngitis, we can assume a population from which these data were randomly extracted Now if there is another set of data from a new patient such as

$$x_0(x_{01},x_{02},\ldots,x_{0\alpha},\ldots x_{0\beta},\ldots x_{0k})$$

we would like to see whether or not this patient belongs to a pre established population such as laryngitis It is known (Torii et al 1965) that if the parent population has a normal distribution, a function F_s defined by

$$F_s=\frac{(N-K)N}{k(N+1)}\sum_{\alpha=1}^{k}\sum_{\beta=1}^{k}\phi_{\alpha\beta}(x_{0\alpha}-\bar{x}_\alpha)(x_{0\beta}-\bar{x}_\beta) \quad (3)$$

has an F distribution with its degrees of freedom determined by k and N where the $\phi_{\alpha\beta}$ are constants which are implicitly defined by the following set of linear equations

$$\sum_{\beta=1}^{k}\Phi_{\alpha\beta}\phi_{\beta\gamma}=\delta_{\alpha\gamma},\ \alpha,\gamma=1,2,\ldots,k \quad (4)$$

where $\delta_{\alpha\gamma}$ is the Kroeneker delta

$$\delta_{\alpha\gamma}=\begin{cases}1 & \text{if}\quad \alpha=\gamma\\ 0 & \text{if}\quad \alpha\neq\gamma\end{cases} \quad (5)$$

and where the coefficients $\Phi_{\alpha\beta}$ in eq 4 are defined explicitly in terms of the parameter values of the data sets as

$$\Phi_{\alpha\beta}=\sum_{i=1}^{N}(x_{i\alpha}-\bar{x}_\alpha)(x_{i\beta}-\bar{x}_\beta) \quad (6)$$

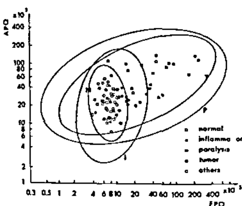


Fig 2 Location of the subjects on the FPQ versus APQ plane *Abscissa* FPQ on a logarithmic scale *ordinate* APQ on a logarithmic scale Solid curves depict the critical ellipses at the 5% level of significance

If an appropriate value such as 5% or 1% for the level of significance θ is selected and a corresponding value of $F_0=F_{N^*k}(\theta)$ is obtained from the F tables, then by comparing F_s with F_0 it can be determined at the given level of significance, whether or not the data set from the new patient belongs to the same population previously assumed It is this determination that constitutes a most important part of the present processing It is an improvement over the use of the Euclidean distances between the means of pairs of assumed populations Obviously, the borderline for this determination is a surface where $F_s=F_0$, which defines an ellipsoid in the k dimensional space Although only two dimensions (FPQ and APQ) were employed in the present study, the efficiency of the analysis may be improved by increasing the number of dimensions, i e, relevant parameters, in future investigations

RESULTS

Both quotient values revealed remarkably skew (exponential) distributions Since the significance of the "parametric' statistical procedures depends upon the mathematical characteristics of the normal (Gaussian) distribution the accuracy of statistical decisions will be diminished if the distribution of the data deviates from the Gaussian pattern It was thought imperative, therefore, to perform an appropriate transformation on the data in order to match the type of distribution to the normal distribution, in advance to the application of the critical ellipse technique A logarithmic transformation ($Y=10\log_e X$, where X represents the value of FPQ×1000 or APQ×1000) was made for both indices for this reason

Fig 2 shows the location of the data points for both normal and pathologic subjects on the FPQ versus APQ plane for their comfortable phonations The axes are scaled logarithmically to reflect the variable transformation described above, even though the scales still show the actual values of the FPQ and APQ It is seen that normal subjects occupy a rather limited area, while the pathologic cases disperse over a wide range of values It is interesting to note that there are some areas specific to certain categories, though a considerable overlap of the areas for different categories is observed The logarithmic scaling permits the use of normal statistics, for which the critical regions become ellipses, as is also shown in Fig 2 The curves shown indicate the critical ellipses for each category at the 5% level of significance

If the level of significance (namely the magnitude of type 1 error) is to be smaller, then the size of the ellipses would be larger and the differentiation among various subject categories would become more difficult, since

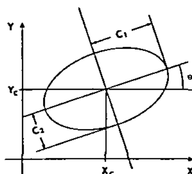


Fig 3 An arbitrary ellipse showing the definition of the parameters used in Table III

Table III. *Constant values for the equations of critical ellipses for each of the 4 homogeneou subject categories for the 5% level of significance*

Subject group	Center (X_c, Y_c)	Rotation θ	Semi major axis C_1	Semi minor axis C_2	F_0
Inflammation	(20 10, 29 95)	77 8°	22 07 [33 42]	13 11 [19 84]	5 79 [13 27]
Paralysis	(28 98, 37 48)	17.5°	37 41 [60 15]	19 39 [31 16]	4 74 [12 25]
Tumors	(30 32, 38 04)	24 7°	32 60 [42.53]	13 74 [17 92]	3 59 [6 11]
Normal	(19 52, 30 91)	−81 7°	14 62 [18 66]	7 14 [9 10]	3.33 [5 42]

the F_0 values at the 1% level are greater than those at the 5% level On the other hand, the size of the ellipses for these pathologic groups would become much smaller if more patients became available, since the F_0 values for these groups decrease in inverse proportion to the increase in the number of subjects Table III shows the F_0 value for each subject group as a reference

Fig 3 illustrates an arbitrary ellipse together ith the geometric parameters which define it e center of the ellipse has coordinates (X_c, Y_c), the tilt of the major axis with respect to the x-axis is the angle θ, C_1 is the length of the semi-major axis, and C_2 is that of the semi-minor axis Table III gives the values of these constants for the 5% critical ellipses [1% values in the square brackets] for each of the homogeneous categories shown in Fig 2

DISCUSSION

Normal and pathologic status

There seem to be some prerequisite considera tions which should be made before any dis- crimination between normal and pathologic subjects is attempted First of all it should be questioned whether or not there exists a *per se* borderline between normal and pathologic voices Some people who have no appreciable pathology in the larynx may produce hoarse voices which in turn reveal considerable varia tions in terms of acoustic measures such as pitch perturbations There may be many pa tients with certain laryngeal diseases on the other hand, who do not reveal appreciable vocal disturbances until a considerably ad vanced stage of involvement It is apparent that the concept of voice quality, which in cludes hoarseness, should be distinguished from the concept of the physical status of the larynx, which includes pathological involve ments, even though these two concepts are undoubtedly closely related to each other It seems justifiable to mention that the existence of a pathology in the larynx does not necessa rily differentiate a pathologic voice from a nor mal voice It also seems fairly obvious that the axis for voice quality is a continuum between the normal and abnormal extremes, and that any borderline differentiating abnormal from normal voices has to be defined from an opera tional point of view

Many previous authors give a *range* of measured values from some selected normal subjects as a normal standard, and regard this as defining a borderline This type of naive norm is quite popular in many clinical jour nals Others have presented a "normal con trol' against some pathologic data They have

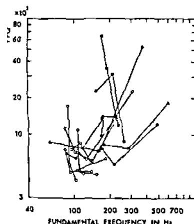


Fig 4 The effect of the fundamental frequency on FPQ in limited normal and pathologic subjects *Ordinate* FPQ on a logarithmic scale, *abscissa* the fundamental frequency in Hz on a logarithmic scale Symbols indicating the type of subject are the same as in Fig 2 The utterances produced by the same subject are connected by a solid line No simple relationship is seen to exist between the two variables, though the FPQ often assumes a lowest value for a "comfortable pitch for a given subject

selected normal subjects whose age and sex "match' with the pathogic subjects being studied, so that the difference in the measured index values between the normal and the pathologic subjects may be attributable to the existing pathology, rather than to other differences such as sex and age Although this type of control is more sophisticated than the naive norms mentioned above, the logical conclusion from such a control study may be simply that the selected pathologic cases could be differentiated from the selected normal controls No normative data for a normal population becomes available from an intentionally selected set of controls

A complete random sampling of the population of normal subjects to establish statistically meaningful normal limits, on the other hand, is also hard to accomplish A random sampling design for various pathologies is even more difficult We therefore have to assume a conceptual population for each homogeneous subject group, such as normal, cancer, etc, from which the available data have been randomly extracted In other words, by regarding the subjects (who have been drawn as random ly as possible without adding any intentional bias) as a set of "random like" samples it is feasible to assume certain populations which are of empirical significance The limits or borderlines of the acoustical measures employed in the present study, therefore were defined for each homogeneous subject group operationally, on the basis of the available data The focus of consideration then was to determine, at a given level of significance, whether or not a newly presented subject falls into one of the homogeneous populations defined above This is not the same as describing a "typical" or "ideal" sample of which a given population consists The two-way discrimination between the normal population and the entire population of pathologic cases was not attempted here since the set of "abnormal" subjects as a whole did not seem to represent a homogeneous population

Effects of fundamental frequency, intensity and different vowels

Although the linear effect of the overall fundamental frequency of the utterance upon the frequency perturbations was accounted for by normalizing, there may be some secondary effect remaining This effect was studied by plotting the FPQ against the fundamental frequency for a limited set of normal and pathologic subjects As is easily imagined, control of vocal pitch was often lost in patients with laryngeal pathology, and in many cases the pitch ranges were rather limited Fig 4 shows the FPQ plotted against the fundamental frequency The utterances by the same subjects are connected by solid lines No simple relationship is seen to exist between these two variables It would seem noteworthy, however, that the FPQ often assumed its lowest value for the "comfortable" pitch for a given subject, and higher values for higher or lower pitch utterances That is, in some subjects phonations in "uncomfortable" or forced pitch levels resulted in appreciable perturbations

No apparent relationship was found be-

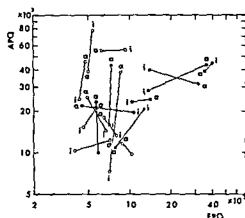


Fig 5 Vowel /a/ and vowel /i/ produced by limited normal and pathologic subjects on the FPQ versus APQ plane Symbols are the same as in Fig 2 Utterances by the same subject are connected by a solid line The locations and shifts of the data points are seen to be quite random

tween the APQ and fundamental frequency either A tendency toward higher APQ values in 'uncomfortable' pitch levels was observed in some subjects, although others did not reveal such an inclination

The intensity, or, more appropriately, "loudness" of phonation as subjectively controlled by the speaker, was found to reveal no invariant effect on FPQ or on APQ within e range of the present data Although the surement of intensity was not made and erefore this effect was not systematically pursued, the available data seemed to indicate that the variability of the quotients due to loudness difference is smaller than the effect of other phonatory environments such as fundamental frequency

Fig 5 reveals the effect of different vowels (/a/ and /i/) on FPQ and APQ for a limited set of normal and pathologic subjects The utterances by each speaker are connected by solid lines The locations and the shifts of the data points on the FPQ—APQ plane are seen to be quite random and there is no obvious trend attributable to the difference of the vowels /a/ and /i/ The same holds true with other vowels tested It seems justifiable to consider that the difference of vowel does not appreciably affect the rate of variability of the fundamental period and peak amplitude

Some causative mechanisms for pitch and amplitude variability

The results of the present study apparently support the previous authors (Lieberman 1963, Koike, 1969) who indicated that pitch or amplitude perturbations are sensitive to laryngeal pathologies The basic mechanisms for producing such variabilities in the acoustic signals, however, are not yet sufficiently known, as mentioned earlier A series of high speed motion pictures of the larynx analysed in the present study appears to disclose some facts in relation to this mechanism

Fig 6 demonstrates a segment of utterance produced by a patient with a large laryngeal polyp The top trace indicates the contact microphone signal simultaneously registered with the laryngeal parameters shown in the remaining four traces It is interesting to note that the perturbations observed in the ampli

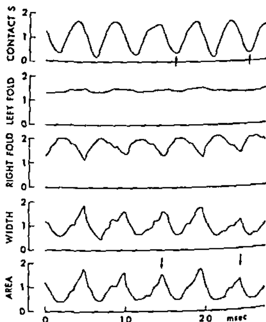


Fig 6 A simultaneous display of a contact microphone signal (*top trace*) lateral excursions of the left (*2nd trace*) and the right (*3rd trace*) vocal folds transverse d ameter of the glottis (*4th trace*) and the glottal area as functions of time The speaker is a patient with a large laryngeal polyp It is seen that a decrease in the peak amplitude of the contact microphone signal (↑ mark) corresponds to a lowering of the area amplitude peak (↓ mark) The ordinates are in arbitrary linear units and the abscissa is time in msec

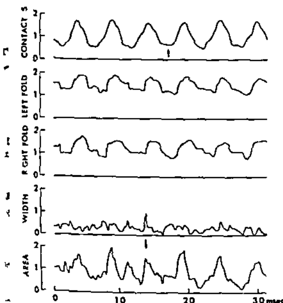


Fig 7 A similar multiple-channel display of the acoustic and physiological parameters The speaker is a patient with laryngeal cancer The lowering of the contact microphone signal (amplitude perturbation ↑ mark) in the top trace is seen to correspond to that in the glottal area waveform (↓ mark)

tude of the contact signal (↑ mark) do reflect the variability of the glottal area waveforms (↓)

This type of agreement between the perturbation of the contact microphone signal and that of the glottal area function was observed frequently in various pathologic speakers Fig 7 for instance, shows another example of a similarly analysed display of the acoustic and physiological parameters for a patient with laryngeal cancer The decreased amplitudes of the minima (amplitude perturbation) of the contact microphone signal (↑ mark) are seen to correspond to those in the glottal area wave form (↓)

These examples illustrate a close relationship between the acoustic signal and the glottal area function, and may in turn justify the use of acoustic measures for extracting the pathologic information related to the glottal area waveform It is not as yet clear, however how such a change in the glottal area function is brought about by the existence of the pathology in the larynx

Our data seem to indicate considerable complexity in this particular mechanism In Fig 6, for example, the left vocal fold with a large polyp reveals little vibration while the other, healthy fold vibrates almost periodically, and the short-term decrease of the amplitude (amplitude perturbation) corresponding to that of the area wave seems to be present in the contour of the healthy, right fold, rather than in that of the affected, left fold

An acceptable explanation for the area wave variability in the cancer case illustrated in Fig 7 also seems to be difficult Since both vocal folds of this patient vibrated nearly 'in phase', and a small but relatively regular "phase lag" between the two folds was responsible for the area periodicity, however, it is conceivable that minute variations in this phase angle between the folds are yielding the area wave variability This in turn may be attributable to the difference in the physical characteristics such as mass, tension or shape of the two folds

Fig 8 illustrates this mechanism fairly explicitly Although in this case the acoustic signal

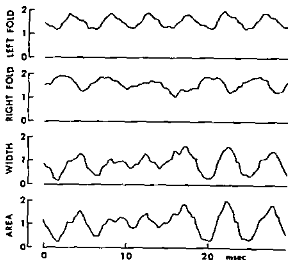


Fig 8 An utterance by a patient with unilateral recurrent nerve paralysis The glottal area wave reveals a marked perturbation of pitch and amplitude corresponding to the discrepancy between the lateral excursion contours of both folds This discrepancy is apparently based on the differing rate of vibration for each vocal fold

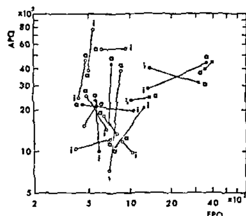


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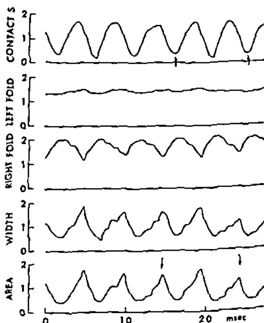


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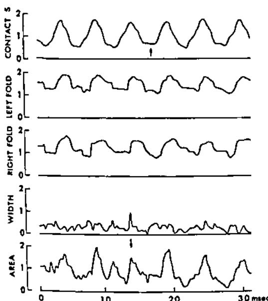


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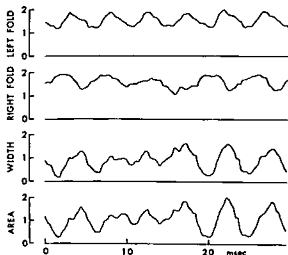


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is not available, the glottal area time function is seen to reveal a marked pitch and amplitude perturbation This irregularity, then, is accounted for by the different rates of vibration for each vocal fold This fact apparently supports Moore & Thompson (1965) who emphasized the independence of each fold

It is apparent, however, that more detailed analyses of pathological data are needed in order to completely elucidate the pathophysiological mechanisms producing various irregularities in the glottal area waveform Indeed, though the display program adopted in the present study uses only one data point to represent the excursion of each fold for example, many more data points may have to be employed to describe adequately the vibration contours of the pathologic folds, since the mode of vibration of the affected fold may be quite complicated

It may not be feasible, furthermore, to study in detail *all* possible pathologic mechanisms related to the area variability, as patients may not be readily available for research in sufficient numbers One of the possible alternatives, then, may be the use of simulation That by simulating various pathological condi either virtually on the excised larynx, theoretically on a simulated model of the ibration in the computer, it should be possible to study the effect of various pathologies upon the glottal area waveform

The efficiency of such study, however, will be dependent upon the applicability of such a model to the real, physiological structure That is, the quality of knowledge derived from such a simulation study will be determined by how closely the physiological structure is imitated On the one hand the data from such simulation study may have to be interpreted rather carefully and conservatively On the other hand, more detailed physiological data related to the laryngeal structure should be collected in order to improve such a (physical or theoretical) model The data obtained from simulation studies should then be examined against the physiological facts according as the accurate and detailed physiological information on the larynx becomes available

Although the physiological data given in the present paper are still limited, it is hoped that the effort of combining physiological facts with the acoustic characteristics of speech as was attempted here may prove helpful to future investigations related to pathologic speech production

ACKNOWLEDGEMENTS

The authors are indebted to Professor Minoru Hirano a Kurume University, Japan for the use of the high speed motion picture apparatus

ZUSAMMENFASSUNG

Storungen in der Grundtonhohe und der Amplitudenscheitelpunkt des mit einem Kontaktmikrophonsystem hergeleiteten akustischen Signals wurden zum Zwecke der Entwicklung nutzbarer Maßnahmen zur Entdeckung laryngealer Pathologie untersucht Einundsechzig Patienten mit verschiedenen laryngealen Pathologien und vierunddreißig normale Versuchspersonen standen unter Beobachtung In vierzehn Fallen wurden mit Filmkamera schnell belichtete Aufnahmen vom Kehlkopf gemacht um den physiologischen Mechanismus zu prufen der die Storungen im akustischen Signal verursacht Der gesammelte akustische Befund wurde statistisch bearbeitet und für jede Fachkategorie wurde eine kritische Ellipse berechnet Die Bedeutung des Begriffes normaler Maßstab wurde besprochen Man wies darauf hin daß der physiologische Mechanismus in den akustischen Storungen ziemlich kompliziert ist und viele physiologische Aspekte mitspielen können Die Notwendigkeit grundlegender physiologischer Forschung in bezug auf pathologische Formen der Spracherzeugung wurde hervorgehoben

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TRACHEOPATHIA CHONDRO OSTEOPLASTICA

A Clinical Study of Thirty Cases

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Abstract During the last 12 years 30 cases of tracheopathia chondro-osteoplastica have been diagnosed at the Department of Otolaryngology of Kuopio University Ten of these were accidentally revealed by bronchoscopy 2 by autopsy but 18 were revealed through a systematic examination Ten of these 18 were preliminarily diagnosed *by indirect laryngoscopy The average age for women* was 51 and for men 42 the youngest patient being 11 and the oldest 71 years of age The characteristic symptoms were long term recurrent cough hoarseness and periodic *expectoration The sputum was frequently abundant and* crusty and sometimes contained streaks of blood Shortness of breath was a common symptom but there were often entirely asymptomatic periods The disease begins h a persistent purulent tracheitis which probably ng to calciphylaxis causes accumulation of calcium in the tracheal mucosa Cartilage and bone later elop around these accumulations In most of the cases of tracheopathia chondro-osteoplastica in the present series the condition was associated with atrophic rhinitis or pharyngitis As the nasal disease improves some regression may occur though hardly healing Calcium and phosphorus metabolism was not disturbed and no immunological aberrations were found in any of the patients in this series

Tracheopathia chondro osteoplastica (TCO) is a rare condition, in which metaplasia of the squamous epithelium occurs in the mucous membrane of the trachea accompanied by accumulation of calcium phosphate and growth of cartilage and bone in the subepithelial connective tissue These appear as hard *nodules in the mucous membrane of the* trachea The number and size of the nodules show great variation They do not occur in the posterior wall Apart from the trachea, the nodules may even spread to the main bronchi but very seldom to the segmental bronchi Large nodules may cause dyspnoea, but the most characteristic symptoms are cough and hoarseness caused by recurrent tracheo-laryngitis, associated by crusty sputum which is often streaked with blood The disease may even be entirely asymptomatic

The first cases of TCO were described as early as 1855 by Rokitansky and in 1857 by Wilks (Dalgaard, 1947) Since then nearly 300 cases have been published The early reports are based mainly on autopsy material, but the majority of the cases published during the last two decades have been identified clinically The most extensive autopsy series, consisting of 20 cases, has been published in Italy (Ragaini & Piccoli, 1957), whereas the largest series revealed by bronchoscopy, consisting of 9 cases, was published in Czechoslovakia (Privara & Cerny, 1965) Most published reports describe only a few cases The geographical spread of TCO is interesting For instance in the United States, Britain and Japan it is probably rare, while being more common in Italy and France By 1972, 30 cases had been published in France (Besso et al , 1972) On the other hand, the rarity of the disease may *depend on the interest shown in its identification* (Way, 1967)

Despite numerous studies, the aetiology and pathogenesis of TCO still remain unexplained

Table I *Age and sex distribution of 30 patients with tracheopathia chondro-osteoplastica*

Sex	Age in years 10–19	20–29	30–39	40–49	50–59	60–69	70–	Total
Female	1	1	2	4	6	5	3	22
Male	–	3	1	1	1	2	–	8
Total	1	4	3	5	7	7	3	30

Dalgaard (1947) made a thorough and commendable study of this disease on the basis of its own case and the literature published up to that time According to him, the undifferentiated connective tissue cells develop into cartilage cells through true metaplasia in the internal layer of the elastic connective tissue, i e in the surface layer of the submucosa and lamina propria Later on these cells lose their immature character, calcium salts accumulate in them and the cellular interspaces, and the calcified cartilage starts forming bone tissue Initially, areas of hyalinization are found, confined to the subepithelial connective tissue of the trachea The infective or metabolic acidosis that first induces this hyalinization process has been considered a primary cause of TCO (Hempel & Glaser, 1958) If these areas remain for a longer period they may become saturated with calcium salts Usually, however, these areas heal and leave slight scars which also may later become calcified In order to elucidate this, Hempel & Glaser investigated a control series of 250 "normal" tracheas, among which they found 11 cases of calcification (4 4%) Hyalinization areas may cause formation of bone and cartilage in the local mesenchymal tissue Cartilage islets formed in the same way may later ossify endochondrally Ossifications may also occur in the ecchondromas of the tracheal rings These ossifications may fuse into those in the submucosa The fact that no bone and cartilage forms in the posterior wall of the trachea, but only in the lateral and anterior walls, is, according to Soulas et al (1971), due to the inducing effect of the cartilage

According to Moser & Them (1954), at the beginning of the century, van Eicken and also Schroter already drew attention to the connection between ozaena and TCO During recent years, attention has also been paid to this relationship, among others by Jepsen & Sorensen (1960), Eimind (1964) and Vaheri & Vaheri (1967) The part played by atrophic rhinitis in cases of TCO in materials such as autopsy material and temporary bronchoscopic findings, has rarely been demonstrated Soulas et al have emphasized that, as the symptoms of ozaena become milder with age, ozaena can escape diagnosis, if the patient is not directly asked about it They drew attention to the metaplasia of the squamous epithelium in the trachea of their patients with ozaena and regarded it as the first symptom of a developing TCO They have described a case in which tracheitis and metaplasia of the squamous epithelium in the trachea were revealed 7 years prior to diagnosing TCO Moser & Them also reported a case in which TCO had been preceded by ozaena of the trachea of 10 years' duration

MATERIAL

At Department of Otolaryngology of Kuopio University 30 cases of TCO have been diagnosed during the last 12 years Ten cases have been accidentally detected in bronchoscopies performed because of symptoms of tracheitis, but 18 cases have been verified when looking systematically for TCO in patients with atrophic rhinitis during the last 10 years Two additional cases revealed at autopsy have been included

Ten of the cases could be preliminarily diagnosed, even by indirect laryngoscopy This is possible when a powerful head light is used At the examination the patient's head has to be bent forward slightly, thus providing a free field of vision in the mirror longitudinally down to the bifurcation Even slight aberra-

Table II *Average age on diagnosing TCO Anamnestically established average age at appearance of atrophic rhinitis and symptoms of tracheitis*

Sex	Average age on diagnosing TCO	Average age at appearance of tracheitis	Average age at appearance of ozaena
Female	51 (22 cases)	39 (16 cases)	25 (15 cases)
Male	42 (8 cases)	32 (7 cases)	25 (6 cases)
Total	47 (30 cases)	37 (23 cases)	25 (21 cases)

tions in the tracheal surface are easy to detect, but because small accumulations of pus on the tracheal wall may erroneously appear in the mirror like TCO nodes, tracheoscopy is essential in confirming the diagnosis In advanced cases with abundant nodes, diagnosis made by endoscopy is easy and reliable, even without biopsy If, again, TCO nodes are few and small, the condition may easily remain unnoticed Nodes must be identified before introducing the bronchoscope If the scope is, in the meantime, advanced towards the bifurcation and drawn up again, the resulting [illegible] a in the mucosa makes it impossible [illegible] slight changes In one case only were [illegible] nodes so large that the introduction of [illegible] caused difficulties Generally, the nodes were small, with a diameter of 1 to 3 mm In more than 10 cases their diameter was 1 mm at the most and often they were located at such a distance from one another that they would probably not have been found in routine bronchoscopy or at autopsy

Twenty eight cases have been confirmed by histo-pathological examination The occurrence of bone and cartilage particles beneath the squamous or normal ciliated epithelium, as well as calcifications in the submucosa, were regarded as positive findings In one patient a specimen could not be obtained for technical reasons In another patient, the nodes were so small and so far apart from one another that the specimen did not include any nodes containing either cartilage or bone but only normal respiratory and squamous epithelium

Age and sex

The present series consists of 8 men and 22 women The youngest female patients were 11, 21, and 37 years of age, while the youngest male patients were 20, 24 and 28 years old respectively On diagnosing TCO, the average age for men was 42 and for women 51 Half of the patients were over 50, the oldest being women of 70 and 71, and a man of 67 The age distribution is shown in Table I

Symptoms

The characteristic symptoms were a recurrent persistent cough, hoarseness, and occasional abundant expectorations which were often crusty and sometimes also streaked with blood Shortness of breath was also a general symptom The majority of patients were occasionally entirely without tracheal symptoms for months and even years

Bacteriological findings

The history of every second patient indicated current or previous purulent tracheitis In 6 of these patients tracheoscopy revealed a purulent and crusty tracheitis, i e the so called tracheaozaena Cultures from their trachea

Table III *Results of cultures from nasal cavity and trachea*

Bacteria	Nasal cavity	Trachea
Staph aureus	6	3
Strept pneumoniae	2	3
Enterobacter aerug	2	2
E coli	1	3
Klebs pneumoniae	3	3
Klebs ozaenae	–	4
Proteus sp	2	5
Diphtheroid	–	2
Str non haemol	–	1
Str viridans	1	–
Str β-haemol group A	–	1
Haem influenzae	1	1
Pseud aeruginosa	1	1
Alcaligenes faecalis	1	
Mycobact tuberc	–	1

exhibited the following bacteria *Klebsiella ozaenae* in 3 cases and *Klebsiella pneum* + *E coli* *E coli*, and *Proteus vulg* +*Staph aureus* in one case each The results of the other cultures are shown in Table II

Age at the onset of symptoms

Because most of the patients had had respiratory symptoms long before TCO was diagnosed, efforts were made to establish anamnestically when the symptoms had appeared According to this clarification, symptoms of tracheitis had appeared in women approximately at the age of 39 and in men at 32 From memory, the symptoms of atrophic rhinitis had appeared in both sexes approximately at the age of 25 (See Table III)

Clinicochemical and immunological examinations

The laboratory tests revealed no aberrations characteristic of TCO patients The serum calcium and phosphorus values were normal for the 20 patients examined In 12 cases, serum urate and alpha 1 antitrypsin were also normal No anaemia occurred and the white blood pictures were within the normal ranges In 13 patients, serum immunoglobulin A, G and M as well as c^3 and c^4 were examined and found normal For a rough estimation of cell bound immunity, the Mantoux test was performed on some patients, but an increased sensitivity could not be demonstrated Thus, no immunological processes could be demonstrated in these patients

Other diseases

Atrophic rhinitis or pharyngitis was diagnosed in 23 patients, 6 men and 17 women In four cases the nose was not examined and only 3 noses proved to be entirely healthy at the examination, one of these patients had had a severe episode of hay fever when she was young One of the patients with healthy noses had had pulmonary tuberculosis a few years earlier The nasal cavities of some older women appeared to be almost asymptomatic though they had been afflicted with ozaena in their youth In one case, the crusty character of the nose was due to Rendu Osler disease

Follow-up

After the initial examination, 7 patients could be followed up for 15, 10, 10, 5, 4, 4, and 3 years respectively In the patient with the longest follow up, a woman aged 65, a purulent tracheitis was diagnosed 10 and 2 years before TCO occurred In a male patient with a 10-year follow-up and TCO diagnosis at the age of 24, a crusty tracheitis was also verified 4 years prior to diagnosing TCO Since diagnosis, the condition had remained the same in the former patient, whereas in the latter, it had progressed with recurrent tracheitis, and nodes in the trachea have increased in number and size during the last 6 years No complete healing was observed, although it was obvious that the changes eased in 2 cases, but in the majority of the cases followed up the changes remained the same

DISCUSSION

The laboratory tests suggest that patients with TCO have no general and continuous disorder in their calcium and phosphorus metabolism, nor could any immunological disorder be demonstrated Likewise, clinical examination has not revealed any common general disease On the other hand, 23 out of 30 had atrophic rhinitis or pharyngitis This indicates that these patients may have an aberrant local mode of reacting to respiratory infections and proneness to formation of bone in the mucosa of the trachea Many of the patients also had tracheaozaena and, to all appearances, half the patients at least had had it at an earlier stage

Selye (1962, 1970) has produced extensive publications on calciphylaxis and calcergy In neither of these phenomena have blood born antibodies or other indications of a hypersensitivity been verified However, calciphylaxis is an artificially induced condition of hypersensitivity in which tissues react to a suitable

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Male	42 (8 cases)	32 (7 cases)	25 (6 cases)
Total	47 (30 cases)	37 (23 cases)	25 (21 cases)

tions in the tracheal surface are easy to detect, but because small accumulations of pus on the tracheal wall may erroneously appear in the mirror like TCO nodes, tracheoscopy is essential in confirming the diagnosis In advanced cases with abundant nodes, diagnosis made by endoscopy is easy and reliable, even without biopsy If, again, TCO nodes are few and small, the condition may easily remain unnoticed Nodes must be identified before *introducing the bronchoscope If the scope is,* in the meantime, advanced towards the bifurcation and drawn up again, the resulting [illegible] in the mucosa makes it impossible [illegible] slight changes In one case only were [illegible] so large that the introduction of [illegible]onchoscope caused difficulties Generally, the nodes were small, with a diameter of 1 to 3 mm In more than 10 cases their diameter was 1 mm at the most and often they were located at such a distance from one another that they would probably not have been found in routine bronchoscopy or at autopsy

Twenty-eight cases have been confirmed by histo pathological examination The occurrence of bone and cartilage particles beneath the squamous or normal ciliated epithelium, as well as calcifications in the submucosa, were regarded as positive findings In one patient a specimen could not be obtained for technical reasons In another patient, the nodes were so small and so far apart from one another that the specimen did not include any nodes containing either cartilage or bone but only normal respiratory and squamous epithe lium

Age and sex

The present series consists of 8 men and 2[illegible] women The youngest female patients wer[illegible] 11, 21, and 37 years of age, while the younges[illegible] male patients were 20, 24 and 28 years ol[illegible] respectively On diagnosing TCO, the aver age age for men was 42 and for women 51 Half of the patients were over 50, the oldes[illegible] being women of 70 and 71, and a man of 67 The age distribution is shown in Table I

Symptoms

The characteristic symptoms were a re current persistent cough, hoarseness, an[illegible] occasional abundant expectorations whic[illegible] were often crusty and sometimes also streake[illegible] with blood Shortness of breath was also [illegible] general symptom The majority of patient[illegible] were occasionally entirely without trachea[illegible] symptoms for months and even years

Bacteriological findings

The history of every second patient indicated current or previous purulent tracheitis In 6 of these patients tracheoscopy revealed a puru lent and crusty tracheitis, i e the so-called tracheaozaena Cultures from their trachea

Table III *Results of cultures from nasal cavity and trachea*

Bacteria	Nasal cavity	Trachea
Staph aureus	6	3
Strept pneumoniae	2	3
Enterobacter aerug	2	2
E coli	1	3
Klebs pneumoniae	3	3
Klebs ozaenae	–	4
Proteus sp	2	5
Diphtheroid	–	2
Str non haemol	–	1
Str viridans	1	–
Str β-haemol group A	–	1
Haem influenzae	1	1
Pseud aeruginosa	1	1
Alcaligenes faecalis	1	–
Mycobact tuberc	–	1

durch Calciphylaxisphanomen eine Deposition der Kalziumsalze in der Trachealschleimhaut verursacht Knorpel und Knochen entwickeln sich spater an der gleichen Stelle Verbessert sich der Status der Nase wird vielleicht eine teilweise Rückbildung der Trachealveranderungen vorkommen aber vollige Genesung mag selten sein Keine Störungen des Kalzium und Phosphorstoffwechsels und keine immunologische Storungen wurden festgestellt

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INFLUENCE OF TEMPERATURE AND DECREASED WATER CONTENT OF INSPIRED AIR ON THE CILIATED BRONCHIAL EPITHELIUM

A Physiological and Electron Microscopical Study

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(Received July 20 1976)

Abstract Experiments have been carried out on the bronchi and intrapulmonary airways of rats *in vitro* to determine the physiological effects of air with water content (WC) of 2 19 mg/l (5% RH (relative himidity) 37°C) 8 77 mg/l (20% RH 37°C) 24 13 mg/l (50% RH 37°C) and 30 71 mg/l (70% RH 37°C) on ciliary beat frequency and also the morphological effect of air at temperatures of 30 40 and 48° at a constant WC of 28 0 mg/l on the ciliated epithel um The results showed t (i) ciliary beat frequency decreased with decreasing d irreversible ciliostasis was reached sooner the the WC value (ii) ciliary beat frequency was not icantly affected above 24 mg/l (iii) 45 min and 90 n exposure at 30°C caused an enormous increase in the depth of the mucus film The cilia and the epithelial cells did not differ from the controls at 40°C (iv) in airways exposed for 45 min at 48°C the cilia appeared to be in total disarray Some were clustered together in whirling arrangements while others were detached from their bases The substructure and the cytoplasm of the cells on the surface and in the deeper layers appeared to have been destroyed (v) after 90 min at 48°C the damage to all structures had progressed Only isolated tissue or cytoplasmic elements were percept ble

The effects of humidity and temperature changes on pulmonary function are well documented (Josenhans et al 1969 Cheney & Butler, 1968, Modell 1968 Melville et al , 1970, Melville & Morris, 1972, Millar et al , 1965) Cold air, in addition to causing an increase in airway resistance via a vagal pathway (Melville, 1972), results in drying of the mucosal membrane, leading ultimately to arrest of ciliary activity and mucus flow inflammatory changes, retention of tenacious mucus, bacterial infiltration, and patchy atelectasis (Benson et al , 1966, Dery et al 1967) Hot dry air and hot humid air are both detrimental to the respiratory ciliated epithelium of a patient with defective air conditioning mechanism, as the respiratory epithelium becomes either dehydrated or overheated (Walker & Wells, 1961) Thus, the maintenance of a proper mucociliary function depends, to a large extent on the fine adjustment of humidity and temperature (Iravani 1967) Therefore, it is necessary to know to what extent the physiological, as well as the morphological aspects of the respiratory ciliated epithelium are affected by controlled variations of these factors The literature on morphological changes in the tracheobronchial ciliated epithelium (Mercke 1974 Mecklenburg et al , 1974) due to physical factors, is scant

The present paper reports findings *in vitro* on the effects of relative humidities under 70%, on ciliary beat frequency, as well as the morphological effects of temperatures from 30°–48°C over varying time periods on the respiratory ciliated epithelium of rats

able I *Summary of changes in ciliary beat requency with air of different water contents temp 37°C)*

ater ontent (mg/l)	Duration of exposure (min)	Changes in ciliary beat (frequency/min)	Rehumidification with air of WC (24 mg/l)
2 19	0 1–0 5	80% reduction	Reversible
2 19	2 0–5 0	ciliostasis	Irreversible
8 77	0.5–1 0	35% reduction	Reversible
8 77	8 0	ciliostasis	Irreversible
4 13	120	18% reduction	Reversible
0 71	120	10% reduction	Reversible

METHODS

The experiments were carried out in female Wistar rats weighing between 120 and 150 g The animals were anaesthetized with sodium phenobarbital (40 mg/kg i p) and the airways prepared as previously described (Iravani, 1967) Once the dissection to free the intrabronchial aireays was completed, the tracheal cannula was connected to a water manometer and the intrabronchial pressure raised to 75 mm H_2O The level chosen was dependent on the critical closing pressure of the terminal bronchioles (Iravani & Melville, 1974) The airway was at all times filled with air, thus preventing the entrance of the surrounding Krebs solution (Krebs & Henseleit, 1932) The Krebs solution was equilibrated with carbogen (95% O_2, 5% CO_2)

Ciliary activity was observed through the membranous wall of the intact air filled airway by means of incident light microscopy (Ultropaksystem, Leitz) at 22×10 and 22×25 magnifications (Iravani, 1967) At temperatures above 25°C cinematographic films were taken at frame speeds between 100 and 200/sec These films were replayed at a slower speed, thus allowing the estimation of ciliary beat frequency

The relative humidity (RH) of the air delivered to the airway was reduced by placing a dehydrating agent ($CaCl_2$) in the delivery tube and the relative humidity was measured at the inlet to the trachea by dry and wet bulb thermometry The effect of 5, 20 50 and 70% RH at 37°C on the ciliary beat frequency was studied The delivery tubes were insulated to prevent excessive heat and condensation in the tubes

In other experiments, following d s of the intrapulmonary airways, the preparation was allowed to remain in incubation medium for 45 and 90 min temperatures of 30°C, 40°C and 48°C respectively The absolute water content (WC) was held constant at 28 mg/l The entire preparation was fixed as outlined below

Transmission electron microscopy (TEM)

Pieces of the trachea and bronchi were fixed in a solution of 4% glutaraldehyde in 0 1 M phosphate buffer (pH 7 4) for 12 hours at 4°C After repeated rinses in buffer (pH 7 4), the pieces were cut into smaller specimens for the post fixation in 2% OsO_4 in buffer for 2 hours Then they were dehydrated in ascending strengths of ethanol and embedded in Araldite Ultrathin sections were obtained with a Reichert ultramicrotome (OmU2) and stained with uranyl acetate and lead citrate on copper grids The sections were examined on a Zeiss EM 9S

Scanning electron microscopy (SEM)

Fixation and dehydration were identical as for TEM up to the 70% ethanol stage The small pieces of trachea and bronchi were dried in CO_2 (Andersson, 1951), glued onto copper grids and coated with a uniform layer of evaporated gold Scanning electron micrographs were obtained with a Jeol scanning microscope U 35

RESULTS

In rats there is a location dependency of ciliary frequency in the bronchial tree, for example, in the lobar bronchus the ciliary beat frequency is about 1200/min at 37°C and in the segmental bronchus it is 950

Air with a WC below 24 mg/l (50% RH

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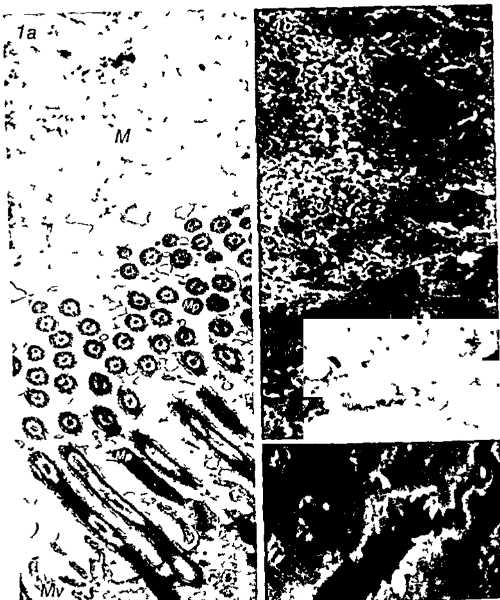


Fig 1 (*a*) TEM of rat bronchi incubated in Krebs solution at 30°C for 45 min Note the thick mucus layer (*M*) covering the tips of the bent cil a The interciliary spaces and the areas around the microvilli of the ciliated cell (*CC*) are devoid of mucus *Mp*=microorganisms (Mycoplasma) in transverse (middle right) and l tudinal section (below) ×24 000 (*b*) SEM of the area The thick mucus film is almost continuous spaces are artefacts caused by the drying ×1000 the same area ×5 000

37°C) appears to be detrimental to proper ciliary function, an effect that is dependent on the duration of exposure to the dry air Air, with a WC of 2 19 mg/l (5% RH 37°C) caused an 80% reduction in ciliary beat fre quency in the bronchi in 0 1–0 5 min This effect was reversible when the water content level was immediately increased above 24 mg/l After 1 5 min at a WC of 2 19 mg/l an irreversible ciliostasis occurred Air, a WC of 8 77 mg/l (20% RH, 37°C) slo ciliary beat frequency by 35% in the minute and caused irreversible cilios after 8 min At 24 mg/l (50% RH 37°C more ciliary beat frequency was not sig cantly affected Exposure for up to 2 h did not result in ciliostasis These results summarized in Table I



Fig 2 (*a*) TEM 30 C 90 min A goblet cell (*GC*) containing mucigen granules (*MG*) can be seen extruding the apical part of its cytoplasm into the less thick mucus film (*M*) The electron transparent spaces around the microvilli (*Mv*) of the goblet cell (*GC*) and the ciliated cell (*CC*) is probably the area occupied by the interciliary fluid ×13 500 (*b*) TEM 30°C 60 min The mucigen synthesizing apparatus—endoplasmic reticulum (*ER*) and Golgi complexes (*Go*)—almost totally occupies the developing GC Here three goblet cells can be seen clustered together and have apparently been induced to produce mucigen by the cold stimulus *L* lysosome ×13 500

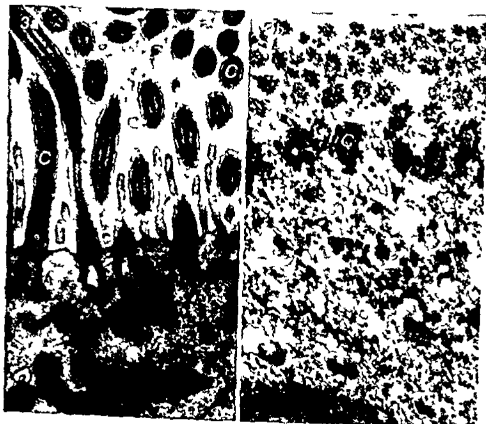




Fig 3 (*a*) TEM of the apical part of a ciliated cell under normal conditions i e the animal was fixed by perfusion immediately after anaesthetization Note the normal structure of the bent cilia (*C*) in longitudinal and transverse sections ×24 000 (*b*) TEM 48°C 45 min The substructure of the cilia and the ciliated cell has been destroyed Abbreviations as in Fig 3 ×24 000 (*c*) SEM 48°C 45 *min* *The* cilia are clustered together and are partially detached from their base The surface of the cilia displays wart-like projections a pathological effect of the heat ×15 000

The dependency of ciliary beat frequency temperature has already been published (Iravani & Melville, 1976) In the bronchi Q10 for ciliary beat frequency is between and 4 5

microscope results

Airways incubated at this temperature used as controls, since rats have a temperature in this region After 45 min were no noticeable alterations com- with airways fixed immediately after the animal (Fig 3*a*) Mucus produc ion was not influenced The cilia and micro- villi remained unaltered The myelinated and myelinated fibres of the laryngeal nerve were well preserved and were also no dif- from those of airways immediately ed after killing the animal No demon- e changes were seen over an exposure of 90 min

°C The most significant change after 45 min exposure at this temperature, was the large quantity of relatively thick mucus layer (approx 3 5 μm) lying on the tips of the cilia in an almost uninterrupted layer (Fig 1*a*) The cilia appeared normal The thick mucus layer prevented observation of the erlying epithelium in scanning electron icrographs and evaluation of the state of ation of the goblet cells was there - not possible (Fig 1*b*) Transmission on microscopy showed that most of the et cells had extruded their mucigen to form mucus

After 60 min, TEM showed that the goblet having extruded their products, had re- ult the mucigen synthesizing apparatus as denced by the increased endoplasmic lum and numerous Golgi complexes (Fig 2*b*) After 90 min, TEM showed that apical parts of fully developed goblet is containing new mucigen granules were n the process of being extruded (Fig 2*a*) A thick mucous sheath is again visible The crovilli of the cells were surrounded by a transparent space probably representing interciliary fluid (Fig 2*a*) The mucigen granules were of varying density signifying varying degrees of hydration

48°C Low magnification SEM of airways incubated at this temperature for 45 min showed that most of the cilia were lying in an irregular arrangement Many were clus tered together while others were detached from their bases The surface of the cilia appeared rough and they seemed to adhere to each other (Fig 3*c*) The mucus film was not preserved The substructures of the cell organelles were completely broken up (Fig 3*b*)

After 90 min the destruction was more extensive and only the collagenous fibres of the connective tissue seemed to be intact, although they had been separated from their connection

DISCUSSION

Mucociliary function in the tracheobronchial tree depends not only on the body tempera ture, but also on the temperature and relative humidity of the inspired air The appara tus responsible for mucus secretion is more sensitive than the ciliary mechanism to changes in temperature and humidity, in that ciliary beating continues even after mucus secretion has ceased (Dalhamn, 1956) According to Yeager (1971) mucus transport is influenced by the strength and frequency of the ciliary beat and the relative thickness of the mucus laycer

In this study, air with decreased water content at constant temperature and air of varying temperature at constant water content, were studied for their effects on the ciliated epithelium

Decreased ciliary activity was observed with a decrease in absolute humidity and the degree of damage was dependent on the length of exposure Proetz (1933) found that ciliary activity was unaffected at relative

humidities of 70% and above, whereas at 50 and 30% ciliary activity ceased in 5 and 10 min respectively A water content in the inspired air below 24 mg/l led to an almost immediate slowing of ciliary activity and the irreversible ciliostasis is linked with alterations in the rheological characteristics of the mucus Richards & Marriott (1974) found that a decrease in relative humidity caused an increase in the viscosity of the mucus The increase in mucus viscosity is probably due to a loss of water from the mucus resulting in a closer packing of the macromolecules and thus favouring an increase in the number of secondary bonds between them Such a situation could influence the ciliary beat frequency A decrease in ciliary activity was observed with decreasing temperature (Mercke et al , 1974) or with an increase in temperature above 42°C (Iravani & Stein hausen, 1967, Iravani, 1967) Further increases in temperature led to a dramatic decrease in beat frequency and to irreversible damage ultimately leading to ciliostasis at 46°C (Mercke et al , 1974) Iravani (1968) found that temperatures as low as −4°C had noticeable effect on ciliary activity for ods of up to 1 hour The ciliary function uld possibly be more resistant to colder emperatures than the mucus secreting apparatus At 30°C in the present study a significant increase in depth of the mucus layer was observed This increase in the mucus layer could be the result of the cooler air directly stimulating the goblet cells to secrete more mucus in order to protect the underlying epithelium from thermal damage This theory gains support from the increased amount of granulated endoplasmic reticulum and Golgi complexes and in our opinion is indicative of increased synthesis *In vivo* such a situation is conceivable if the irritant receptors located in the trachea (Filenz & Widdicombe, 1972) were to respond by increasing mucus production in the lower air ways, thus producing sufficient moisture for humidification of the incoming dry air

At 48°C the cilia adhered at their tips and all cellular organelles were destroyed At moderate heat exposure Mecklenburg et al (1974) found submembranous vesicles and fusion of the tips of the cilia and considered them to be cellular defence mechanisms Barber & Boyde (1968) considered them to be artefacts and Dahlgren et al (1972) as toxic reactions In our opinion, the increase in temperature probably leads to a partial alteration of some cellular enzymes and, due to the increase in cellular metabolism and the change in the enzymes, the byproducts of metabolism are not rapidly deactivated, thus leading to further cellular injury

These findings clearly indicate that improper conditioning of the inspired air could lead to morphological alterations in the ciliated epithelium and a reduction in the physiological functioning of the cilia Andersen et al (1974) exposed healthy subjects to air at 23°C and 9% RH (WC 2 1 mg/l) for 78 hours and concluded that there was no physiological need for humidifying the inspired air This picture is different in patients with respiratory diseases or in those where for one reason or another the upper respiratory tract is bypassed It is in these patients that the proper adjustment of temperature and relative humidity is of prime importance

ZUSAMMENFASSUNG

In vitro-Experimente werden an Bronchien und intrapulmonalen Atemwegen von Ratten unternommen um die physiologischen Auswirkungen der Luft mit verschiedenen Wassergehalten (WC) auf die Zilienschlagfrequenz zu bestimmen Des weiteren werden die morphologischen Veranderungen am Flimmerepithel durch Atemluft mit Temperaturen von 30 40 und 48°C bei einem konstanten WC von 28 mg/l beschrieben

(1) Die Zilienschlagfrequenz verringert sich mit abnehmenden WC, und irreversible Ciliostasis tritt in umso kurzerer Zeit ein je niedriger der WC ist (2) Oberhalb 24 mg/l wird die Zilienschlagfrequenz nicht signifikant beeinflußt (3) Der Einfluß von Luft mit einer Temperatur von 30°C bewirkt nach 45 und 90 Minuten ein beträchtliches Anwachsen des Schleimfilms Die Zilien wie das Epithel unterschieden sich nicht von Kontrollversuchen bei 40°C (4) In Atemwegen die 4

Minuten bei 48°C gehalten wurden legten sich die Zilien in vollkommene Unordnung Einige schienen miteinander verklebt und waren in Wirbeln angeordnet andere waren von ihren Basen gelost Die Substrukturen der Organellen sowohl der Zellen an der Oberflache wie in tieferen Schichten schienen zerstort (5) Nach 90 Minuten bei 48°C war die Zerstorung aller Strukturen fortgeschritten Nur einzelne Gewebe und Zytoplasmaelemente waren erkennbar

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FIRING PATTERN OF MOTOR UNITS IN THE VOCAL MUSCLE DURING PHONATION

F Šram and J Syka

From the ENT Department of the Postgraduate Medical and Pharmaceutical Institute and the Institute of Experimental Medicine Czechoslovak Academy of Sciences Prague Czechoslovakia

(Received August 12, 1976)

Abstract Electromyographic activity of the vocal muscle was analysed in 10 subjects during the phonation of different vowels and with different voice pitches Analysis of the firing pattern of single or a few motor units was performed with the aid of an amplitude discriminator on the computer The firing rate increases with increasing voice pitch (in the region $c_0 - h_0$ the decrease of mean interspike interval with the slope 30–40 msec/octave was found) In the firing pattern of several motor units the periodicity was observed to be correlated with the voice pitch The period of 8 msec corresponding to H 123 z decreased to about 6 msec during phonation of d_0. Hz The periodicity was present both in units with prephonatory activity and in units which were active g phonation It seems possible that the activity in the al muscle changes characteristically during the phonation of different vowels

Since the paper by Weddel et al (1944), introducing into otolaryngology the method of recording the electrical activity of intrinsic laryngeal muscles, vast amounts of data have been accumulated regarding the function of laryngeal muscle (e g Katsuki, 1950 Portmann et al , 1956, Faaborg Andersen, 1957, Buchtal, 1959, Wustrow & Wieck, 1963, Buchtal & Faaborg-Andersen, 1964, Hirano et al 1969, Hirano et al , 1970, Gay et al 1972 Hirose & Gay, 1972) EMG of laryngeal muscles has been used since the late 1940s as a valid tool in establishing the diagnosis in various voice disorders especially concerning the size and location of the lesion in the neuromuscular system Usually the EMG data are evaluated from the paper records or oscilloscopic displays, in which case the overall EMG activity from clusters of motor units recorded with the aid of needle electrodes is the main source of information However, a more elaborate analysis of the laryngeal EMG is possible only when the activity of a single motor unit or a few units is processed Some data obtained in this way have already been published by Sutton et al (1972), who described mainly the average frequency of firing in cricothyroid muscles, their spontaneous and evoked activity It is a well established fact that the tension of the vocal muscle affects the voice pitch and that the characteristic features of a vowel arise from the configuration of the supralaryngeal space (Faaborg Andersen & Vennard, 1964) However, it is still not clear, to what extent the activity of the vocal muscle changes during the phonation of single vowels

It was the purpose of this study to isolate from the EMG activity spikes of either one or a few motor units of the vocal muscle and to perform on the computer analyses of such spike trains with different statistical methods The main questions were how is the average firing rate correlated with the voice pitch and with the type of vowel, does any periodicity in the firing pattern of motor units exist and if it does how is it correlated with the voice pitch and with the type of vowel?



Fig 1 Relationship of the mean interspike interval and the voice pitch irrespective of the type of vowel *Ordinate* Mean interspike interval (in msec) *abscissa* voice pitch ▲ units with pure prephonatory activity + units active both during prephonation and phonation phase O units which are also spontaneously active *Note the* decrease in the mean interval within frequencies marked by interrupted lines

The electrical activity of vocal muscles was recorded simultaneous with the patient s voice and with the physician s notes on the multi channel frequency modulated tape recorder

For the purpose of a quantitative evaluation of the EMG the data have to be digitalized i e the form of each potential has to be neglected and the motor potential has to be further processed as a uniform pulse The amplitude served for the selection of motor potentials the potential with an amplitude higher than the preselected level triggered a shape circuit and thereafter only the sequence of selected spikes was evaluated Statistical analysis of the data was performed on the Linc computer (Digital Equipment Corporation) and programs for the evaluation of biological spike trains were used

MATERIAL AND METHODS

The EMG activity of 10 subjects (5 male 5 female) age range 20–62 years was examined Two subjects were volunteers 8 were patients with intermittent weak voice disorders (usually a voice fatigue, without any objective pathological findings) Electrical activity from single or both vocal muscles was recorded with concentric monopolar needle electrodes and amplified by means of DISA A 1430 three channel electromyograph Insertion of electrodes through the skin and cricothyroid membrane was used The subjects obtained two tablets containing belladonna and 50–100 mg phenobarbital before the EMG examination The mucous membrane of the pharynx and larynx was carefully anaesthetized with 0 5% Tetracain solution Patients were instructed to pronounce 30–40 times the same vowel of a certain pitch and with the same loudness during each phonation period

During the experimental session the EMG was monitored on the screen of the DISA Electromyograph and when necessary the electrode position was corrected to select electrical potentials from a single or a few motor units

RESULTS

From the total number of 5495 electromyograms of phonations of single vowels 3473 were selected for the computer analysis the remainder were discarded after a thorough visual analysis of the control paper records (on the basis of technical artefacts high interference of the activity etc)

First the mean interval value was calculated together with its standard deviation in one series of phonations of the same vowel Values of 10 to 160 msec were obtained with the predominance of mean interval between 20 and 80 msec i e with the mean frequency of firing 12 5–50 spikes/sec (Fig 1) Calculated values for each spike train were compared with the type of vowel and the voice pitch No essential correlations were found between the mean interval and the type of vowel however a decrease of the mean interval accompanied the increase in the voice pitch (Fig 1) In the area marked by interrupted lines the decrease is evident significant on the 1% level of confidence with the slope 30–40 msec/octave Mainly units with phonatory and spontaneous activity (crosses and circles) are concerned in the mean interval decrease On the other hand

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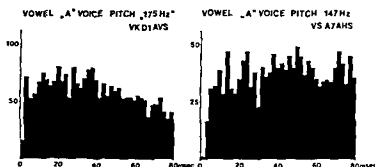


Fig 2 Autocorrelation histograms of the motor unit activity constructed for series of 30–40 utterances of the same vowel with a constant voice pitch (*Left*) Vowel A pitch 147 Hz unit with prephonatory activity (*Right*) Vowel A pitch 175 Hz unit with prephonatory and spontaneous activity *Abscissa* time (in msec) *ordinate* occurrence of spikes

the mean intervals of units with a pure prephonatory activity do not change substantially with the increasing voice pitch (triangles) The latter fact, however, is caused artificially by the mode of calculation of the mean interval, as all intervals, i e even the long intervals between the prephonation periods (when the unit is silent) are summed

Furthermore, the mean interval units were characterized by the distribution of interspike intervals, interval histograms were therefore constructed As regards the type of interval histogram, two main types were distinguished units with regular firing, usually active during ...on or spontaneously active, were ... by a symmetrical type of interval his... with the modal value between 40 and ... msec ($n=11$) The activity of these units usually increased during phonation, though no clearcut correlation was found between the type of vowel or the voice pitch and the modal value The second type of interval histogram was characterized by a quasi exponential distribution of interspike intervals the activity being irregular Mainly units with pure prephonatory activity or with the combination of prephonatory and phonatory activity belonged to this group

The questions arose to what extent was the firing of units of the second group accidental, and is there any periodicity in the firing pattern? For the solution of this question the method of construction of autocorrelation histograms was adopted As a rule autocorrelation histograms with the bin width 2 msec and total duration 80 msec were constructed In total 131 autocorrelation histograms were analysed in 10 patients, constructed from 30–40 utterances of the same vowel

Fig 2 shows two examples of an autocorrelation histogram On the left side, with the histogram corresponding to utterances of the vowel A, with the pitch 147 Hz, the unit was active only during the prephonation period on the right side the histogram is constructed from phonations of the vowel A with the pitch 175 Hz (in another subject), the unit with prephonatory and spontaneous activity It is evident from the figure that regular peaks and dips appear in the autocorrelation histogram

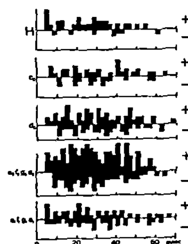


Fig 3 Occurrence of peaks and dips in individual autocorrelation histograms classified according to the voice pitch *Ordinate* (in each of five graphs) Number of peaks (+) and dips (−) *abscissa* time in the autocorrelation histogram (in msec) bin width 2 msec On the left side are marked the frequencies of the voice

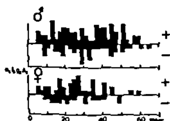


Fig 4 Occurrence of peaks and dips in the autocorrelations histograms of the voice pitch e_0, f_0, a_0 differentiated according to male voices (*top*) and female voices (*bottom*) Further details as in Fig 3

during the first 40–50 msec period For example in the histogram on the left side peaks are present at 4, 12, 20, 28, 38 msec, whereas in the right histogram peaks appear at 14, 24, 38, 50 msec and a dip at 30 msec Occurrence of peaks and dips indicates that in the firing pattern of laryngeal motor units a periodicity is present A similar periodicity was found in the majority of phonations, where interspike intervals were distributed quasi-exponentially

In order to evaluate all autocorrelation histograms by a simple method and to extract from the material the essential features, corresponding to the periodicity code of the voice pitch and probably also of vowels, the following procedure was used autocorrelation histograms were subjected to a thorough visual examination and each peak substantially exceeding the basic level of the histogram was marked 'plus one' and each dip substantially lower than the basic level was marked 'minus one' In resulting graphs obtained by this method, values from different phonations and from different persons were summed together Fig 3 summarizes the data classified according to the voice pitch It is evident that a tendency exists to reduce the period (which is indicated by the length of interpeak or interdip intervals) with increasing voice pitch For example, where the voice pitch equals the tone H (i e 123 Hz), the period is 8 msec, diminishes toward c_0 at d_0 (147 Hz) reaching about 6 msec Due to a lack of data for a single tone, the data for tones e_0, f_0, g_0 and a_0 have been summed In this case, an accumulation of peaks and dips appeared in the same classes of graph (mainly in classes longer than 20 msec), which was caused by interference of periods belonging to single tones on the one hand and by interference of the data from male and female voices on the other Fig 4 shows that when the data were divided in the same graph according to male and female voices far more consistent periodicity was present The period for the male voice is shorter, probably because the head register takes part in the formation of the higher voice pitch, whereas the female voice under such circumstances (voice pitch e_0–a_0) uses only the breast register The longer period thus corresponds to the relatively low pitch level for the female voice

Individual autocorrelation histograms were evaluated by means of the same method for vowels, i e peaks and dips occurring in autocorrelation histograms were summed according to the vowel and irrespective of the voice pitch Fig 5 shows the results, which are less convincing than in the case of the voice pitch In vowel A, where sufficient data were available for the analysis, a remarkable periodicity with an interval 8 msec was present The periodicity was less consistently expressed in other vowels, especially in the case of the

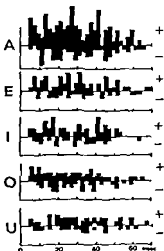


Fig 5 Occurrence of peaks and dips in autocorrelation histograms differentiated according to vowels The type of vowel is marked on the left side Further details as in Fig 3

vowels O and U It seems that the period for the vowel E will be shorter than 8 msec and also the configuration of peaks and dips in the case of the vowel I seems to be different, probably with the participation of lower periods (15–30 msec) The small amount of data in vowels other than A is a serious limitation for any definite conclusion Probably also the loudness of the voice plays a part in the frequency and firing pattern of discharging motor units during phonation However, the great interindividual variability in the voice intensity made it impossible to correlate the data from different persons in the present study

DISCUSSION

In this research work two remarkable features of the activity of motor units in the vocal muscle have been confirmed the frequency of activity (at least in heavier registers) increases with increasing voice pitch and in some units a periodicity is present in the firing pattern which also depends on the voice pitch The periodicity changes, probably during the phonation of different vowels Definite proof of possibility is lacking, however—at least to now The slope of decrease in the mean interval with increasing voice pitch was estimated to be 30–40 msec/octave

The essential features of the EMG laryngeal activity during phonation are known from earlier works (e g Katsuki, 1950, Portmann et al , 1956, Faaborg Andersen, 1957, Buchtal, 1959, Wustrow & Wieck 1963, Buchtal & Faaborg-Andersen, 1964, Hirano et al , 1969, Hirano et al , 1970, Gay et al 1972 Hirose & Gay, 1972) The contribution of our work lies in a quantitative description of the data and in concentrating on the activity of a single motor unit or a few units A similar methodological approach was used by Sutton et al (1972), the subject under investigation being the crico thyroid motor units In comparison with crico thyroid units, motor units in the vocal muscle display higher firing rates (10–15 spikes/sec vs 12 5–50 spikes/sec) Furthermore the U shape function (Sutton et al , 1972) of the correlation between the firing rate and the voice pitch was not observed among units of the vocal muscle The periodicity in firing rate correlated with the voice pitch was described earlier by Portmann et al (1956) and used by Husson (1962) in the explanation of function of laryngeal motor units during phonation. However, due to a lack of quantitative data, the results were subject to critical discussion Our data, which originate from an extensive material and utilize the method of autocorrelation histograms (which analyses periodicity very sensitively), are in good agreement with the data of Portmann et al (1956) In fact the voice pitch is strictly correlated with the period in the firing pattern of a motor unit e g 8 msec period (i e 125 Hz) is present, when the voice pitch was H (123 Hz), at d_0 (147 Hz) it diminished to about 6 msec The sensitivity of the method substantially decreased at higher voice pitch levels, thus it was not possible to estimate exactly the correlation at frequencies higher than 200 Hz However, the distribution of peaks and dips in Fig 3 (at frequencies e_1–a_1) suggests that the period diminishes again to about 4 msec

The objection that the periodicity arises from the microphonic effect of phonation is hardly tenable First, only spikes which sufficiently exceeded the present level (and thus also the background noise level) were processed and, secondly, the periodicity was also found in units with pure prephonatory activity thus in the period preceding the occurrence of the voice sound Thus it may be assumed on the basis of our data that the voice pitch is coded centrally and at least in some frequency regions the periodicity in the firing rate of motor units in the vocal muscle is correlated with the tension of the vocal cords The participation of proprioceptive activity during phonation cannot be excluded either

It remains to be judged to what extent similar code in the vocal muscle takes part in the phonation of single vowels From our results it may be assumed that even single vowels

differ in the periodicity of the firing of motor units The contemporary concept regarding the formation of vowels emphasizes the participation of supralaryngeal resonance spaces, which are effectively influenced by extrinsic laryngeal muscles (Faaborg Andersen & Vennard, 1964) It cannot be excluded, however, that the formation of different vowels is accompanied at least to some extent by the modification of the vocal cord configuration, which may be correlated with the change in the activity of intrinsic laryngeal muscles

ZUSAMMENFASSUNG

Die elektromyographische Aktivitat des Musculus vocalis wurde bei 10 Personen wahrend der Phonation verschiedener Vokale mit verschiedener Stimmhohe analysiert Das Innervationsmuster einzelner oder mehrerer motorischen Einheiten wurde mit Hilfe eines Amplitudendiskriminators auf dem Computer analysiert Die Innervationsfrequenz nimmt mit steigender Stimmhohe zu (im Bereich von c_0-h_0 betragt die Abnahme des zwischen den einzelnen Aktionspotentialen liegenden mittleren Intervallen 30-40 msec/Oktave) Beim Innervationsmuster mehrerer motorischen Einheiten wurde eine mit der Stimmhohe in Bezug stehende Periodizitat beobachtet wobei sich die Periode von 8 msec die H 123 Hz entsprach wahrend der Phonation von d_0 147 Hz auf etwa 6 msec verminderte Diese Periodizitat erschien dann sowohl in Einheiten mit reiner Praphonationsaktivitat als auch in Einheiten d e wahrend der Phonation aktiv waren Es ist daher möglich daß sich die Aktivität im Musculus vocalis wahrend der Phonation verschiedener Vokale charakteristisch verandert

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PURULENT AND NON PURULENT MAXILLARY SINUS SECRETIONS WITH RESPECT TO pO_2 pCO_2 AND pH

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Abstract Antral secretions from patients with maxillary sinusitis were aspirated for determination of pO_2 pCO_2 and pH In 14 non purulent secretions the mean pO_2 was 12 7 kPa (96 mmHg) and the mean pCO_2 5 2 kPa (39 mmHg) The mean pH was slightly alkaline In 18 purulent secretions usually with a heavy growth of pneumococci or *H influenzae* the pO_2 was zero or close to zero and the mean $pCO_2 \geq 10$ 1 kPa (≥ 76 mmHg) The mean pH was slightly acid and significantly lower than in the non purulent secretions The gas composition and the pH in purulent secretions do not only influence the metabolic activity and multiplication of bacteria but may also interfere with the local protective functions of the sinus osa and with the bactericidal function of granulocytes

e composition of the antral air changes during maxillary sinusitis Thus Aust & Drettner (1974*a*) showed that the oxygen tension was lower in patients with acute maxillary sinusitis than in normal subjects and that the oxygen content of the antral air was related to the patency of the maxillary ostium The alterations in the gas composition within the sinus may be of pathophysiological importance in sinusitis as the mucosa consumes oxygen from the antral cavity (Aust & Drettner, 1974*b*) Whether this consumption of oxygen is of vital importance for the mucosal functions, such as the ciliary activity is not yet established (Reimer & Toremalm) However in empyemic infection of the maxillary sinus the antral cavity is filled by retained secretion to a greater or lesser extent The gas composition in the retained secretion is therefore of considerable interest, especially as the bactericidal function of the granulocytes is oxygen dependent (Nathan & Baehner, 1971)

This study was undertaken to determine the partial pressure of oxygen (pO_2) and carbon dioxide (pCO_2) of retained sinus secretion in cases of maxillary sinusitis In addition the pH values of the secretions were determined as the hydrogen ion concentration is of vital importance for bacterial growth and the functioning of mucosal cells In particular we were interested in the possible differences between purulent and non purulent secretions as regards pO_2, pCO_2 and pH as these secretions represent different forms of inflammatory condition (Boyd, 1963) Such differences in pO_2 pCO_2 and pH may reflect both the difference in curability and prognosis, and also indicate the selection of treatment

MATERIAL AND METHODS

Sampling procedure and bacteriological examination

From 28 untreated patients (22 female and 6 male, aged 21 to 71 years) suffering from maxillary sinusitis with symptoms of 30 days duration at the most, 32 samples of sinus secretion without macroscopical admixture of blood were aspirated through a Lichtwitz needle introduced into the sinus through the

Table I *The reproducibility of the electrode responses*

pO_2 and pCO_2 determined in two gas standards and in saline and maxillary sinus secretion saturated with the gas standards. The mean values and the standard deviations of 10 measurements are given in kPa

	Gas standard			
	I		II	
	pO_2	pCO_2	pO_2	pCO_2
Gas standard	11.8±0.1	4.9±0.1	0±0.1	9.9±0.2
Saline	11.9±0.4	4.7±0.2	0±0.2	10.0±0.4
Secretion	11.8±0.4	4.7±0.1	0±0.2	10.0±0.6

inferior nasal meatus. Syringes of glass were used. Immediately after aspiration the secretions were classified as purulent or non purulent secretion. Secretions mainly of low viscosity, opaque and discoloured were classified as purulent. The non purulent secretions were either serous (i.e., clear, pale yellow secretions of low viscosity, frequently with the capacity to form a gel), or mucous (i.e., grey or brownish semiopalescent secretions of high viscosity). In all, 18 purulent and 14 non purulent secretions were obtained (Tables II and III).

The secretions were examined for bacterial growth by the conventional agar plate technique in routine use at the Department of Clinical Microbiology of Karolinska sjukhuset, and also by immediate inoculation of the secretions into broth agar bottles (prepared by the National Bacteriological Laboratory), one for aerobic and one for anaerobic growth. The bottles were incubated (37°C) within 12 hours. Furthermore, the occurrence of and the morphology of the bacteria were examined on smears stained by the Gram method.

Immediately after the aspiration of secretion the syringe was sealed by inserting the needle into a rubber stopper. Within a few minutes the sample was analysed for pO_2 and pCO_2. Samples with air bubbles were discarded.

Determination of pO_2, pCO_2 and pH

In the determination of pO_2 and pCO_2 in sinus secretion an Ultra Micro Gas Analyzer, IL 113, (Instrumentation Laboratory Inc., USA) was used together with a Constant Temperature Control Modul, IL 127, providing a constant temperature environment (37°±0.5°C) for the pO_2 electrode (IL 17026), the pCO_2 electrode (IL 16050) and the measuring chamber. These electrodes are modifications of the Clark O_2 electrode and the Stow CO_2 electrode (Severinghaus & Bradley, 1958). pH was determined with a separate pH-meter (IL 175, pH-electrode IL Lot-403 30-M8).

For the determination of pO_2 and pCO_2 in secretions, the measuring chamber (0.1 ml) was filled from a syringe. To avoid the trapping of air bubbles within the chamber a sample volume of 0.5 ml was injected through the chamber in 2–3 doses during one minute. Furthermore, before introduction of the sample, the electrode system was equilibrated with a gas standard whose composition was similar to the expected value of the sample. In this way the effect of remaining gas dissolved in the electrolyte was minimized.

Calibration

The electrode assembly was calibrated daily taking into consideration the actual atmospheric pressure and the vapour pressure of water (37°). Two gases of known composition (5.1%±0.1% CO_2, 12.3%±0.1% O_2 in N_2 and 10.5%±0.1% CO_2 in N_2, respectively), the gas standards, were used in the calibration procedure. The slope standardization of the pCO_2 electrode assembly was performed with these gas mixtures and the gas analyser was balanced to provide a scale read out from 10 to 100 mmHg (from 1.3 to 13.3 kPa).

Before every sample determination the gas analyser was adjusted to the actual calibration values.

Reliability

To equilibrate liquids with the gas standards a tonometer (IL 237) was used. The maximal volume of liquid tonometered was 5 ml and the gas flow through the tonometer (37°C) was adjusted to 300 ml/min.

Table II. *Eighteen purulent secretions aspirated from maxillary sinus*
The results of bacterial cultures, smears, pO_2 and pCO_2 in kPa and hydrogen ion concentrations are shown

Patient no	Bacterial culture	Smear		pO_2	pCO_2	pH
16	Pneumococci	Gram+diplococci		0	11 8	6 9
24	*H influenzae*	Gram−rods		0	9 6	6 9
42	Pneumococci	Not studied		0	7 2	7 0
43	Pneumococci	Not studied		0 3	10 8	7.3
46	*H influenzae*	Not studied		0 7	7.3	7.2
51	*H influenzae*	Not studied		0	≥13.3	6 9
65	Pneumococci	Gram+diplococci		0	≥13.3	6 1
66	Pneumococci	Gram+diplococci		0 3	8 1	6.9
71	Pneumococci	Gram+diplococci		0	≥13 3	6 6
72	Pneumococci	Gram+diplococci		0	10 6	6 4
73sin	Pneumococci	Not studied		0	9 3	6 4
76	Anaerobic micrococci	Gram+cocci, gram+rods		0	≥13.3	5 8
78	Pneumococci	Gram+diplococci		0	5.5	6.8
79	Pneumococci	Gram+diplococci		0	9 2	7 2
83	β-streptococci, *Staph epidermidis anaerobes*	Gram+cocci, gram+rods, gram−rods		0	≥13 3	6 9
86	*H influenzae* Pseudomonas	No bacteria		0	9 3	6 9
87	Pneumococci	No bacteria		0	8 2	7 1
88sin	*H influenzae*	No bacteria		0	7 7	7 3
	Bacterial growth 18/18	Bacteria 10/13	Mean	0 1	≥10 1	6 8
			S D		≥ 2.5	0 4

The response time and the stability of the lectrode were determined with saline tonotered with the gas standards A stable elecode deflection was obtained within one minute The pCO_2 readings and the zero value of pO_2 were stable (−0 1–+0 3 kPa in 10 determinations) for at least 10 min The pO_2 reading (>0 kPa) was stable for at least 2 min (−0 4–+0 7 kPa in 10 determinations) Accordingly, the pO_2 and pCO_2 of the sinus secretions (in this sequence) were measured 1–2 min after introduction of the sample into the chamber

To compare the reproducibility of the electrode responses in gases and liquids, saline and a mucous maxillary sinus secretion were tonometered with the gas standards pO_2 and pCO_2 were determined repeatedly with the gas standards and the liquids in the measuring chamber The differences in reproducibility between gas standards, saline and secretion were small (Table I)

RESULTS

The results of the analysis of the 18 purule secretions are shown in Table II The pO_2 wa in 15 secretions 0 kPa and never >0 7 kP The mean pCO_2 was ≥10 1 kPa with a range o 5 5–≥13 3 kPa and the mean pH was 6 8 wi a range of 5 8–7 3

Bacterial growth was demonstrable in a purulent secretions (Table II) Pneumococ were isolated in 11 secretions and *H influen zae* in 5 secretions In 2 secretions were anaer obic bacteria found The bacteria were ob tained in pure culture in all secretions excep two In smears bacteria were seen in 10 of 13 studied secretions, usually in great numbers

The results of the analysis of the 14 non- purulent secretions are shown in Table III The mean pO_2 was 12 7 kPa with a range of 4 9–20 5 kPa and the mean pCO_2 was 5 2 kPa with a range of 1 3–13 2 kPa The mean pH of the secretions was 7 4 with a range of 6 7– 8 0

Table III *Fourteen non purulent secretions aspirated from maxillary sinus*

The results of bacterial cultures smears pO_2 and pCO_2 in kPa and hydrogen ion concentrat ons are shown
* mucous secretions ** serous secret ons

Patient no	Bacterial culture	Smear		pO_2	pCO_2	pH
27*	Pneumococci	Gram+rods		10 8	6 3	7 4
28*	Pneumococci	Gram+d plococci		17 0	1 3	6 7
36**	No growth	No bacteria		17 4	2 0	7 8
67**	Pneumococci	No bacteria		8 2	8 8	7 2
70**	*Staph ep dermids*	No bacteria		15 2	6 1	7 6
73dx**	Pneumococci	No bacteria		4 9	13 2	7 8
77a**	Pneumococci *Staph ep dermds*	No bacteria		20 5	2 9	7 7
77b*	Pneumococci *Staph ep dermds*	No bacteria		8 2	5 2	7 4
80**	No growth	No bacteria		8 6	5 3	7 3
81a**	No growth	No bacteria		13 4	4 4	7 4
81b**	No growth	No bacteria		16 8	3 2	7 6
84**	*H influen ae*	No bacteria		9 8	6 1	7 0
88dx*	*H influenzae*	No bacteria		10 9	2 9	8 0
89**	No growth	No bacteria		16 0	5 1	7 3
	Bacterial growth 9/14	Bacteria 2/14	Mean	12 7	5 2	7 4
			S D	4 6	3 0	0 3

Bacterial growth was demonstrable in 9 of 14 non purulent secretions (Table III) Pneumococci were isolated in pure culture in 4 secretions and *H influenzae* in 2 secretions *Staphylococcus epidermidis* was found in one secretion and a mixture of pneumococci and *Staphylococcus epidermidis* in 2 secretions In the remaining 5 secretions however there was no growth of bacteria In smears bacteria were seen in 2 of 14 secretions studied

The differences between purulent and non purulent secretions as regards the means of pO_2 pCO_2 and pH were statistically significant ($p<0\ 001$ Student s *t* test)

DISCUSSION

In order to improve the treatment of maxillary sinusitis which still constitutes a therapeutic problem much work has been undertaken to clarify the underlying pathophysiological processes It has been suggested that changes of the gas composition in the sinus may be of importance to the onset and course of maxillary sinusitis (Flottes et al 1960)

In purulent maxillary sinusitis the antral cavity is occupied by retained secretion lying in direct contact with the mucosa and harbouring bacteria Therefore it is of interest to ascertain the pO_2 the pCO_2 and the pH of the secretion as these qualities of the sinus environment are of importance not only to the bacterial growth but possibly also to the function of the mucosa and the granulocytes

The assays of pO_2 pCO_2 and pH of the secretions made it necessary to aspirate the secretions as no pCO_2 electrode is available small enough to be introduced into the sinus by puncture The possible error inherent in the aspiration procedure i e an admixture of atmospheric air to the retained secretion was limited by aspirating rapidly a small part (0 5 ml) of the total volume of the retained secretion (frequently more than 5 ml) The very low oxygen content in the purulent secretions indicate that this error was negligible From the experiments on the reliability of the gas ana

lyser it was found that also mucous secretions with high viscosity could be properly analysed

No reports on the gas composition of maxillary sinus secretion have been found, but in pleural effusions low pO_2 and high pCO_2 have been demonstrated, particularly in purulent effusion (Funahashi et al, 1971)

The gas composition of the antral air in man has been investigated previously Thus, in patients with "chronic maxillary sinusitis" Kitayama (1968) found in gas samples from the antrum "with comparatively good ventilation" and "with little or no ventilation" an oxygen content of 17 29 and 16 60% respectively, corresponding to 16 40 and 15 74 kPa (at an ambient atmospheric pressure of 760 mmHg or 101 kPa) Aust & Drettner (1974*a*) found by introducing a small pO_2-electrode into the maxillary sinus, a mean pO_2 of 116 6 mmHg (15 5 kPa) in normal subjects with patent ostia In subjects with obstructed ostia this value was 88 7 mmHg (11 8 kPa) and somewhat lower, 75 mmHg (10 0 kPa), in patients with purulent discharge In the present study the mean pO_2 in non purulent secretions was of the same magnitude, 12 7 kPa In contrast to these results the pO_2 in the purulent secretions was zero or close to zero

Kitayama (1968) also studied the carbon dioxide content of antral gas samples in patients with chronic maxillary sinusitis Converted to partial pressure (assuming an atmospheric pressure of 760 mmHg or 101 kPa) the pCO_2 was approximately 3 5 kPa whether the ostial function was comparatively good or not In the present study the mean pCO_2 in non purulent secretions was higher 5 2 kPa and in purulent secretions markedly higher, $\geqslant$10 1 kPa

Thus the pO_2 and pCO_2 of the non purulent sinus secretions correspond fairly well with the composition of antral air previously found in diseased sinuses The very low pO_2 and high pCO_2 of the purulent secretions found in the present study may be explained by a difference in number of bacteria between purulent and non purulent secretions In the purulent secretions bacterial growth was found in every case In the non purulent secretions bacteria were isolated in 9 of 14 secretions In smears bacteria were seen in 10 of 13 studied purulent secretions in contrast to the non purulent secretions in which bacteria were seen only in 2 of 14 cases A proper quantification of the bacteria in smears was not possible, although the absence of visible bacteria in spite of bacterial growth probably indicates a smaller number of bacteria in these secretions However, whether the gas composition of the secretions was influenced by the metabolism of the bacteria in the secretions could not be judged from this study

The pH reaction of the secretions varied quite considerably, 5 8–8 0 In purulent secretions the mean pH was slightly acid and significantly lower than the mean pH of the non-purulent secretions which is in agreement with earlier studies (Lundberg & Malmborg 1974)

Changes in the gas composition and hydrogen ion concentration in the maxillary sinus may be of importance to the onset and course of the sinusitis Thus, a prerequisite for bacterial establishment and multiplication within the sinus is a suitable biochemical environment pO_2, pCO_2 and pH are important environmental factors as bacteria have specific requirements in this respect differing from species to species Thus, the growth of pneumococci and *H influenzae* is not impeded in oxygen poor environment, but rather stimulated by the high carbon dioxide tension and the slightly acid pH of the purulent secretion (Cowan & Steel, 1974)

The gas composition within the sinus is also of importance, as the defence mechanisms of the sinus mucosa may be influenced From experiments on dogs, Flottes (1960) concluded that the mucociliary activity is reduced in the sealed-off sinus as an effect of oxygen poor environments Reimer & Toremalm found a decreased ciliary activity in human maxillary sinus mucosa when exposed to

oxygen poor gas *in vitro*. Whether the mucociliary activity *in vivo* is impeded in the extremely unphysiological environment of purulent sinusitis is still an open question. The oxygen exchange through the normal maxillary sinus mucosa is limited by perfusion (Aust & Drettner, 1974*b*), but whether the gas exchange through the swollen, inflamed mucosa is also limited by diffusion is not yet established. The fact that the major part of the adsorbed oxygen is consumed by the mucosa (Aust & Drettner, 1974*b*) may indicate that the mucosa is dependent on oxygen supply from the gas within the antrum.

Another part of the defence system is the bactericidal activity of phagocytic cells. The granulocytes possess several antimicrobial mechanisms, one of which being the oxygen consuming hydrogen peroxide–myeloperoxidase–halide system (Klebanoff, 1968). In some diseases, when no hydrogen peroxide is produced by the granulocytes, catalase-positive bacteria survive and may even multiply within the granulocyte, in contrast to catalase-negative bacteria, which are eliminated. The catalase negative bacteria, such as the aerotolerant anaerobes pneumococci, streptococci and *H. influenzae* produce hydrogen peroxide as part of their normal metabolism and it is believed that this small amount has bactericidal capacity when potentiated by the myeloperoxidase of the granulocytes (Quie 1972, Stossel, 1974). However, in anaerobic environments, such as the purulent sinus secretion, neither the granulocyte nor the bacteria can produce hydrogen peroxide. It is therefore conceivable if this antibacterial mechanism as a potent part of the defence system is inhibited in sinus empyema permitting survival of the catalase negative bacteria.

To conclude, the present investigation has shown that there is a pronounced difference between purulent and non purulent maxillary sinus secretions regarding the oxygen tension in particular, but also regarding the carbon dioxide tension and the pH reaction. The pO_2, pCO_2 and pH found in purulent secretions is consistent with optimal growth of pneumococci and *H. influenzae*, the bacteria most frequently found in maxillary sinusitis (Rantanen & Arvilommi, 1973, Cauwenberge, 1976). However, this environment as regards pO_2, pCO_2 and pH is unphysiological and may be of importance in reducing the function of the local defence mechanisms of the sinus mucosa. Therefore the present results strongly favour the traditional view that purulent sinusitis should be treated by drainage of the sinus cavity, not only to reduce the debris and the number of microbes, but also to improve the condition for the local defence mechanisms of the sinus.

ZUSAMMENFASSUNG

Antrale Sekrete von Patienten mit maxillarer Sinusitis wurden zur Bestimmung von pO_2, pCO_2 und pH aspiriert. In 14 nichteitrigen Sekreten ergab sich für pO_2 ein Mittelwert von 12 7 kPa (96 mmHg) und für pCO_2 ein Mittelwert von 5 2 kPa (39 mmHg). Der mittlere pH Wert war leicht basisch. In 18 eitrigen Sekreten – überwiegend mit starkem Vorkommen von Pneumococci oder *H. influenzae* – betrug der Wert von pO_2 Null oder fast Null, und der Mittelwert von pCO_2 war $\geq$10 1 kPa ($\geq$76 mmHg). Der mittlere pH Wert war leicht sauer und bedeutend niedriger als in den nichteitrigen Sekreten. Die Gaszusammensetzung und der pH Wert beeinflussen nicht nur die metabolische Aktivitat und Vermehrung von Bakterien, sondern konnen auch die örtlichen Schutzfunktionen der Sinusschleimhäute sowie die bakteriziden Funktionen von Granulozyten beeintrachtigen.

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DIE OBERFLACHE DER REGIO OLFACTORIA DES MENSCHEN IM RASTERELEKTRONENMIKROSKOP

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Abstrakt Die Oberflache der regio olfactoria eines 25-jahrigen Mannes wird rasterelektronenmikroskopisch beschrieben Entsprechend den Abschnitten der distalen Rezeptorzellendigungen zeigt die Oberflache unterschiedliche Ebenen

1 Zu oberst ein unterschiedlich dicker Schleimfilm

2 In d[illegible] tronenmikroskopisch mit dem Terminalfilm eine morphologisch faßbare funktionelle Einheit

3 Es folgt die Schicht der kurzen basalen Riechharchenabschnitte Diese gehen uberwiegend sternförmig von den olfactorischen Vesikeln ab (5–12/Vesikel) und tauchen nach kurzem Verlauf bogenförmig in den Terminalfilm ein

4 Daran schließt sich die Ebene der olfactorischen Vesikel (Ø etwa 1 μm) an mit überwiegend kolben teilweise auch keulen- und pyramidenförmigem Aussehen

5 Es folgt die Etage der Mikrozottenrezeptoren mit etwa 100 und mehr Zotten pro Zelle

6 Zu unterst finden sich die dicht aneinandergelagerten apicalen Stutzzellenoberflächen die eine unterschiedliche Große und einen regelmaßig angeordneten Mikrovillibesatz aufweisen

Aufgrund des etagenförmigen Aufbaus der Oberflache des Riechepithels und insbesondere des relativ uniformen Aussehens der distalen Riechharchenabschnitte könnte aus rastermikroskopischer Sicht angenommen werden daß die geruchswirksamen Molekule unmittelbar mit der rasterelektronenmikroskopisch faßbaren funktionellen Einheit von Terminalfilm und dicht aneinander gelagerten distalen langen Riechhärchenabschnitten reagieren so daß für ein unterschiedliches Geruchsempfinden eher eine unterschiedliche Struktur bzw Anzahl von geruchswirksamen Molekülen als eine unterschiedliche Form der distalen Rezeptorzellendigungen in Erwägung zu ziehen ist

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Oberflächenstrukturen der Regio olfactoria sind von Graciadei & Trucker bei der Wasserschildkrote (1970), Bertmar bei der Forelle (1972), Adams bei der Maus (1972), Lawry beim „Lantern"-Fisch (1973), Lenz beim Kaninchen (1972), Breipohl et al beim Goldfisch (1973) und Andres beim Rhesusaffen und der Bachforelle (1975) beschrieben worden

Ziel dieser Arbeit ist es, über erste rasterelektronenmikroskopische Befunde der regio olfactoria des Menschen zu berichten, um aus rasterelektronenmikroskopischer Sicht zum moglichen Angriffspunkt der geruchswirksamen Molekule Stellung nehmen zu konnen

MATERIAL UND METHODEN

Von einem 25 jahrigen, todlich verungluckten Mann wird drei Stunden nach dem Unfallgeschehen die regio olfactoria einschließlich der angrenzenden Nasenschleimhaut von der lamina cribrosa, den medialen Flächen der Nasenmuscheln und dem oberen Septumbereich entnommen Die gewonnenen Riechschleimhaute werden in 0,9%-iger Kochsalzlosung aufgefangen, mit dieser abgespult und danach in kleinere Stuckchen von einigen Millimetern Durchmesser zerlegt und in 5%-iger gepufferter Glutaraldehydlosung eine Stunde lang fixiert Kurz vor der Fixierung werden bei 9 Praparaten die Oberflachen an einigen Stellen artefiziell ver

Riechhärchen, die olfactorischen Vesikel, die Mikrozottenrezeptoren und die apicalen Zelloberflachen der Stutzzellen darstellen zu konnen Anschließend werden die Praparate in aufsteigender Alkoholreihe entwassert (30, 40, 50, 60, 70, 80, 90, 100 und 100%), wobei die Verweildauer der Praparate in dem betreffen den Alkohol jeweils 1/4 Stunde betragt Aus dem 100%-igen Alkohol werden die Praparate unmittelbar in Amylacetat eingetaucht und mit dieser Losung in die Kritische Punkttrocknungsanlage[1] eingebracht In dieser Anlage erfolgen 5–10 Waschungen mit CO_2 Danach werden die getrockneten Praparate auf runde Metallobjekttrager aufgebracht und mit einer Goldschicht von 50–100 A in der Sputteranlage[2] uberzogen Zusatzlich erfolgt die Anfertigung einiger histologischer Kontrollschnitte Insgesamt werden 20 Riechschleimhaut-Praparate im Rasterelektronenmikroskop[3] angesehen Sowohl die im Rasterelektronenmikroskop sichtbaren Oberflachenbeschaffenheiten als auch die insgesamt 480 rasterelektronenmikroskopisch getatigten Fotos (Große 10×10 cm², Vergroßerungsbereiche 20–15 000 fach, uberwiegend 1 000–7 000 fach) werden ausgewertet

ERGEBNISSE

Terminalfilm (Abb 1)

Alle Praparate sind annahernd vollstandig mit einem Sekretfilm unterschiedlicher Starke uberzogen, so daß die Riechharchen und olfaktorischen Vesikel uberwiegend nicht zu erkennen sind (Abb 1*A*) An einigen Stellen jedoch wo der Terminalfilm weniger stark ausgepragt ist, schimmern Einzelkonturen von Riechhärchen durch (Abb 1*B*) Bei sehr dünnem Terminalfilm wie er an einigen Stellen zu sehen ist sind die Riechgeißeln
deutlich erkennbar (Abb 1*C*) Dieser u
schiedlich dicke Terminalfilm bedeckt nu
zu oberst gelegenen Riechharchen und r
nicht bis zu den olfaktorischen Vesikeln (
3*A*)

Riechharchen (Abb 2)

Nach artefizieller Entfernung des Sekret
wird deutlich, daß die Riechhärchen dich
einander gelagert sind, uberwiegend par
zueinander verlaufen und eine etagenför
Anordnung mit Bildung einer dichten
uns als Riechharchenmatte bezeichn
Riechharchenschicht aufweisen (Abb
Dabei bildet die obere Riechharchensch
mit dem Terminalfilm eine rasterelektro
mikroskopisch morphologisch faßbare f
tionelle Einheit (siehe Abb 1)

Das einzelne Riechharchen ist rund be
einen Durchmesser von 0,3–0,4 µm und v
unterschiedlich große warzenförmige
dickungen auf (Abb 2*B*) Die apicalen E
der Sinnesgeißeln sind leicht kugelartig
dickt (Abb 2*B*) Bei Betrachtung der ge
ten geschlossenen Riechharchenmatte
die unmittelbar mit dem Terminalfilm in B
hung tretende oberste Riechharchenges
flache im uberwiegenden Maße von den
pern der Riechharchen gebildet und nu
geringen Anteil von den apicalen R
harchenenden (Abb 2*A*)

Die Lange der Sinnesgeißeln ist wegen
dichten Aneinanderlagerung und Verflech
schwer abschätzbar Es konnen Längen
zu 30 µm verfolgt werden

Abb 1 Riechepitheloberfläche eines 25 jährigen M
von der medialen Flache der oberen Nasenmuschel
minalfilm unterschiedlicher Starke (*A*) Sehr dicker
minalfilm mit vollstandiger Verdeckung der Riechhär
rechts im Bild freiliegende uberwiegend parallel
laufende Riechhärchen nach arteficieller Beseitigun
Terminalfilms links im Bild REM Bild Vergröße
1 4600 (*B*) Mittelstarker Terminalfilm der d e R
härchen uberzieht An einigen Stellen schimmern E
konturen der Riechhärchen durch REM Bild Verg
rung 1 3600 (*C*) Sehr dünner Terminalfilm durch
die Riechhärchen deutlich erkennbar sind REM
Vergroßerung 1 8900

[1] SDC 900 EX der Firma Bomar
[2] MINI COATER der Firma Commonwealth Scientific Comp
[3] JSM 35 der Firma Jeol






C

Schicht der basalen Riechharchenabschnitte
(Abb 3)

Erst nach partieller arteficieller Entfernung des Terminalfilms und der uberwiegenden Anteile der distalen Riechharchenendigungen werden die olfactorischen Vesikel mit den Abgangen der basalen Riechharchenabschnitte sichtbar Dabei zeight sich ein nahezu sternformiger Abgang der Riechharchen von den olfactorischen Vesikeln wobei diese Riechhärchen überwiegend von der gesamten Oberflache der olfactorischen Vesikel entspringen (Abb 3*A*) An einigen Stellen gehen die Riechharchen nur von der Basis der olfactorischen Vesikel ab, wobei die Oberflache der olfactorischen Vesikel dann frei von Riechhärchen ist Nach ihrem sternförmigen Abgang von den olfactorischen Vesikeln streben die Riechhärchen einen nahezu senkrechten Verlauf in Richtung Terminalfilm an und tauchen in diesen bogenförmig, teilweise nahezu rechtwinklig ein Diese basalen Riechharchenabschnitte weisen einen etwas großeren Durchmesser als die in den Terminalfilm eintauchen den Riechharchenabschnitte auf Die basalen Riechhärchenabschnitte sind frei von Terminalfilm Sie zeigen ebenfalls warzenartige Verdickungen unterschiedlichen Ausmasses und unterschiedlicher Große (Abb 2*B*)

Von einem olfactorischen Vesikel entspringen etwa 5–12 Riechharchen

Ebene der olfactorischen Vesikel
(Abb 3)

Die olfactorischen Vesikel sind uberwiegend kolben, teilweise auch keulen- und pyramidenformig (Abb 3*A*) Sie ragen im uberwiegenden Maße uber das apicale Zellniveau der Stutzzellen und der Mikrozottenrezeptoren hinaus und bilden eine eigene Ebene für sich im Raum An einigen Stellen jedoch sind auch olfactorische Vesikel in Hohe der Mikrozottenrezeptoren und auch im Niveau des apicalen Stutzzellenoberflachenreliefs zu sehen Der Querdurchmesser der olfactorischen Vesikel betragt etwa 1 μm

Ebene der Mikrozottenrezeptoren
(Abb 4)

Zwischen der Ebene der apicalen Enden der olfactorischen Vesikel und der apicalen Zelloberfläche der Stützzellen liegt das Niveau der Mikrozottenrezeptoren Diese zeigen relativ dicht aneinanderstehende, unterschiedlich lange Mikrovilli Die Lange dieser sog Mikrozotten betragt ca 2–3 μm Ihre Anzahl pro Zelle ist unterschiedlich und schwer abschatzbar Es werden bis zu 100 und mehr pro Zelle gezahlt Der Durchmesser der Mikrozotten betragt ca 0,3 μm Die Mikrozotten verlaufen nahezu senkrecht nach oben und biegen im Gegensatz zu den Riechharchen nicht um Die Mikrozotten uberragen deutlich den Mikrovillibesatz der Stützzellen Bei starker Vergrößerung lassen sich kleine warzchenartige Verdickungen an den Mikrozotten erkennen Die apicalen Enden der Mikrozotten sind leicht verdickt Bei den Mikrozotten wird die apicale Oberflache fast ausschließlich von den leicht verdickten apicalen Enden der Mikrozotten gebildet und nicht von der Flache ihrer runden Korper, wie das bei den Riechhärchen der Fall ist

Die apicale Zelloberfläche der Mikrozottenrezeptoren zeigt eine unterschiedliche Große

Ebene der apicalen Stutzzellenoberflache
(Abb 4)

Die apicalen Zelloberflächen der Stutzzellen sind dicht aneinander gelagert und zeigen einen deutlich erkennbaren, relativ gleichformigen Mikrovillibesatz Dabei sind die Mikrovilli überwiegend locker angeordnet, stellenweise jedoch auch sehr dicht Das

Abb 2 Riechepitheloberflache eines 25 jahrigen Mannes von der medialen Flache der oberen Nasenmuschel nach artefizieller Entfernung des Terminalfilms (*A*) Dicht aneinandergelagerte überwiegend parallel und etagenweise übereinander verlaufende Riechharchen die eine dichte Matte bilden REM Bild Vergrößerung 1 5800 (*B*) Das einzelne Riechhärchen ist rund weist einen Durchmesser von etwa 0,3–0 4 um auf und zeigt kleine warzenartige Verdickungen an seiner Oberflache (↑) Die apicalen Enden der Riechhärchen erscheinen leicht kugelartig verdickt (↑↑) REM Bild Vergroßerung 1 28 000

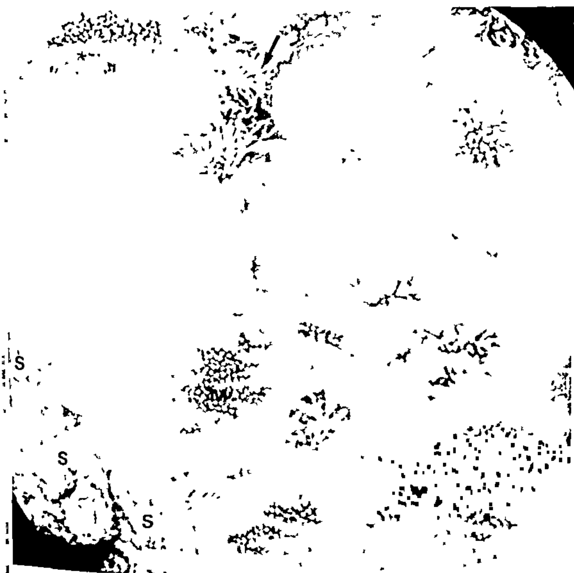


Abb 4 Riechepitheloberfläche eines 25-jährigen Mannes von der medialen Fläche der oberen Nasenmuschel nach ...icieller Beseitigung von Terminalfilm und Riech... am Übergang zur normalen Nasenschleimhaut Mikrozottenrezeptoren mit sehr langen Mikrovilli (↑) Apicale Stützzellenoberflächen unterschiedlicher Größe mit dichtem Mikrovillibesatz (*M*) Restschleim (*S*) Drüsenausführungsgang oben am Bildrand Vergrößerung 1 5000

... 3 Riechepitheloberfläche eines 25 jährigen Mannes von der medialen Fläche der oberen Nasenmuschel nach ...icieller Aufsprengung bzw Beseitigung des überwie... Anteils des Terminalfilms und der distalen Riech...enabschnitte Die kolbenförmigen olfactorischen Vesikel (↑) mit sternförmigem Abgang der proximalen ...enabschnitte (*R*) werden sichtbar Reste des Terminalfilms (*T*) Reste der distalen Riechhärchenabschnitte (*D*) Pyramidenförmiger olfactorischer Vesikel (↑↑) Vergrößerung 1 3700 (*B*) Ausschnittsvergrößerung von (*A*) Kleine wärzchenartige Verdickungen an den basalen Enden der Sinneshärchen werden sichtbar 1 17500

apicale Zelloberflächenrelief der Stützzellen wird an einigen Stellen durch die Öffnungen von Drüsenausführungsgängen unterbrochen, in denen teilweise noch Restschleim liegt

DISKUSSION

Die Oberfläche des Riechepithels zeigt rasterelektronenmikroskopisch vom Terminalfilm

ausgehend bis zur apicalen Zelloberflache der Stützzellen reichend einen Raum, der entsprechend den Abschnitten der distalen Rezeptorzellendigungen in verschiedene, rasterelektronenmikroskopisch morphologisch faßbare und damit gleichzeitig funktionell bedeutsame Ebenen eingeteilt werden kann Gegen die endonasal eingeatmete Luft grenzt ein Terminalfilm unterschiedlicher Stärke Dieser uberzieht die obersten, dicht aneinander gelagerten, uberwiegend parallel zueinander verlaufenden, relativ uniform aussehenden Riechharchen Diese dichte „Riechharchenmatte" bildet zusammen mit dem Terminalfilm eine rasterelektronenmikroskopisch morphologisch faßbare funktionelle Einheit Aufgrund der rasterelektronenmikroskopischen Befunde ist anzunehmen, daß auf diese Einheit die in der Atemluft enthaltenen geruchswirksamen Moleküle unmittelbar auftreffen und zwar zunachst auf den Terminalfilm und nach Passierung desselben unmittelbar auf die dicht aneinander gelagerten Riechharchen Dieser Vorgang vollzieht sich somit stets im feuchten Milieu

Aus rasterelektronenmikroskopischer Sicht scheinen die darunter liegenden Ebenen der oximalen Abschnitte der sternförmig von n olfactorischen Vesikeln abgehenden iechharchen und die Ebene der olfactorischen Vesikel mit unterschiedlichen Formen für einen unmittelbaren Beruhrungspunkt der geruchswirksamen Molekule weniger von Bedeutung zu sein, als die funktionelle Einheit von Terminalfilm und Riechharchenmatte, da ein Durchdringen der geruchswirksamen Moleküle ohne Beruhrung der distalen langen Riechhärchenabschnitte aus rasterelektronen mikroskopischer Sicht nicht vorstellbar ist

Da die etagenförmig angeordneten, dicht aneinanderliegenden Riechharchen ein relativ uniformes Bild zeigen, teilweise mit warzenartigen Verdickungen unterschiedlicher Starke und unterschiedlichen Ausmasses und aus rasterelektronenmikroskopischer Sicht der Angriffspunkt der geruchswirksamen Moleküle an der Oberflache der Riechharchen ein schließlich ihrer apicalen Endigungen statt haben müßte, kame für ein unterschiedliches Geruchsempfinden eher eine unterschiedliche Struktur der geruchswirksamen Molekule neben anderen Faktoren als eine unterschiedliche Form der distalen Rezeptorzellendigungen in Frage Da der Terminalfilm die Riechharchen in unterschiedlicher Stärke uberzieht, konnte es sich moglicherweise auch um eine unterschiedliche Zusammensetzung des Riechschleims bzw um eine unterschiedliche Schleimviskositat handeln Inwieweit die Dicke des Schleimfilms bzw eine unterschiedliche Terminalfilmqualitat fur die geruchswirksamen Moleküle mit ihren oberflächlichen rasterelektronenmikroskopisch sichtbaren Angriffspunkten an den Riechharchen eine Rolle spielen, ware noch zu klaren

Bei den von den Sinnesharchen zu beobachtenden warzchenartigen Verdickungen unterschiedlicher Starke und unterschiedlichen Ausmasses durfte es sich hier aus rasterelektronenmikroskopischer Sicht eher um echte Ausstulpungen als um Auflagerungen handeln Dadurch wird die gesamte funktionelle Oberflache der Riechharchen noch wesentlich vergroßert Inwieweit diese warzenartigen Verdickungen möglicherweise Pradilektionspunkte fur den Angriffspunkt der geruchswirksamen Molekule sein könnten müßten weitere Untersuchungen, insbesondere bei sehr starken rasterelektronenmikroskopischen Vergroßerungen zeigen

Daß die apicalen Enden der Riechhärchen und der Mikrozotten leicht verdickt erscheinen, ist eher auf ein rasterelektronenmikroskopisch praparationstechnisches Phänomen zuruckzuführen, als auf eine mögliche Reaktion der geruchswirksamen Molekule an den apicalen Enden der Sinnesgeißeln Die Lange der Riechhärchen ist wegen der dichten Aneinanderlagerung und dem etagenförmigen Verlauf schwer abschätzbar, wobei riechharchenlagen jedoch bis zu 30–40 μm verfolgt werden können

Die Schicht der proximalen, sternförmig von den olfactorischen Vesikeln abgehenden

Riechharchenabschnitte haben aus rasterelektronenmikroskopischer Sicht wahrscheinlich mehr Stutz- bzw Trager und Reizfortleitungsfunktion, da sie die funktionelle Ein heit von Terminalfilm und „Riechharchen matte' und damit die wesentlich langeren distalen Riechharchenabschnitte abstützen und von der unmittelbaren apicalen Zelloberflache der Stutzzellen abheben Es sei denn, daß die geruchswirksamen Moleküle den Terminalfilm und die Riechharchenmatte durchdringen würden, um an die proximalen Abschnitte der Riechharchen zu gelangen, wobei dann jedoch ein Anstoßen der geruchswirksamen Moleküle an die distalen langen Abschnitte der Riechharchen langst stattgefunden haben mußte Der fehlende Terminalfilm im Bereich *der proximalen Riechharchenab*schnitte, sprache auch etwas gegen eine Reaktion der geruchswirksamen Molekule an diesen proximalen Abschnitten

Die in den Abbildungen erkennbaren kurzen Sinnesgeißeln der olfactorischen Vesikel sind dadurch zu erklaren, daß der weitaus langere Teil der distalen Riechharchenabschnitte arteficiell abgerissen wurde, um die olfactorischen Vesikel überhaupt sichtbar machen zu konnen Ohne willentliche teilweise Artefizierung der Praparate mit ihrer Befreiung vom Terminalfilm und den distalen Riechharchenabschnitten waren die olfactorischen Vesikel nicht sichtbar zu machen

Obwohl die Form der olfactorischen Vesikel teilweise unterschiedlich ist und auch Unterschiede bezuglich des Abgangs der Riechharchen vom olfactorischen Vesikel bestehen, sind ihre Riechgeißeln, wie bereits erwahnt, relativ gleich aussehend Und gerade diese scheinen aus rasterelektronenmikroskopischer Sicht mit den geruchswirksamen Molekulen zu reagieren und nicht die olfactorischen Vesikel da diese tief unter der funktionellen Einheit von Terminalfilm und Riechharchen matte liegen Somit kommt unseres Erachtens der Polymorphie der olfactorischen Vesikel aus rasterelektronenmikroskopischer Sicht eine geringere Bedeutung fur den unmittelbaren Geruchsreizangriffspunkt zu, als den distalen langen Sinnesharchenabschnitten

Die verschiedenen Formen der olfactorischen Vesikel konnten auch aufgrund moglicher De und Regenerationsstadien der olfactorischen Vesikel erklarbar sein (Andres, 1969, Graciadei & Metcalf, 1971, Mulvaney & Heist, 1955, Schultz, 1960)

Beim Vergleich unserer rasterelektronen mikroskopischen Befunde des Menschen mit denen beim Kaninchen und Schaf (Lenz, 1972) stehen ebenfalls die dichtaneinandergelagerten, relativ uniform aussehenden, von einem dichten Terminalfilm uberzogenen Riechharchen im Vordergrund Diese Befunde stehen in gutem Einklang mit unseren rasterelektronenmikroskopischen Befunden am Menschen und denen am Tier von Bertmar (1972), Donald (1972) und Graciadei (1972) Erst nach artifizieller Entfernung des Terminalfilms mit dem uberwiegenden Anteil der distalen Riechhärchenabschnitte konnen auch wir beim Kaninchen wie Breipohl et al (1973) beim Goldfisch nach Entfernung des Termi nalfilms morphologisch unterschiedliche distale Rezeptorzellendigungen feststellen Breipohl und Mitarb lassen es offen, ob diesen morphologischen Varianten entsprechend funktionelle Unterschiede zuzuordnen sind, oder ob diese Formunterschiede der olfactorischen Vesikel möglicherweise aufgrund von De und Regenerationsstadien erklarbar sind Aufgrund unserer rasterelektronenmikroskopischen Befunde sind wir der Ansicht, daß der rasterelektronenmikroskopisch faßbaren funk tionellen Einheit von Terminalfilm und relativ gleichaussehenden dicht aneinandergelagerten Riechharchen die großere Bedeutung für den Hauptangriffspunkt der geruchswirk samen Molekule zukommt, als der unterschiedlichen Form der olfactorischen Vesikel

Bei Selbstversuchen mit elektrischer Rei zung der menschlichen Riechschleimhaut mit dunnen bipolaren, sehr flexiblen Reizelektroden kommt Baumgarten (1975) zu dem Schluß, daß eine Anhäufung von Spezialistenrezeptoren in den einzelnen Gebieten der Riech-

schleımhaut aller Wahrscheınlıchkeıt nach nıcht vorlıegen kann Dıese aus elektrophysıologıscher Sıcht zu vermutende Erscheınung kann mıt unseren rasterelektronenmıkroskopıschen Befunden von der Oberflache der Rıechschleımhaut des Menschen und auch vom Schaf und Kanınchen ınsofern ın guten Eınklang gebracht werden, als daß dıe geruchswırksamen Molekule ıhren unmıttelbaren Angrıffspunkt an der relatıv unıform aussehenden funktıonellen Eınheıt von Termınalfılm und Rıechharchenmatte haben mußten

SUMMARY

This report descrıbes the surface of the regıo olfactorıa of a 25 year-old man as observed ın the scannıng electron mıcroscope Correspondıng to the dıstal ends of the receptor cells the surface dısplays varıous planes (*a*) Uppermost ıs a film of mucus of varyıng thıckness (*b*) The olfactory haırs whıch are arranged closely together mostly ın a parallel fashıon ın layers dıp ınto thıs mucus film The long dıstal parts of the olfactory haırs are of a rather unıform shape and form together wıth the mucus film a sıngle functıonal unıt (c) Now follows a layer consıstıng of the short parts of the olfactory haırs They branch from the olfactory vesıculae mostly ın a stellar fashıon (5–12 on one vesıcula) and arch themselves after a short course ınto the mucus film (*d*) There then follows the plane of the olfactory vesıculae mostly bulbous partly club- and pyramıdal ın shape (*e*) After that comes the lane of the mıcrovıllı receptors whıch have about 100 more vıllı on one cell (*f*) Rıght at the bottom ıs the cal surface of the supportıng cells dıfferent ın sıze wıng mıcrovıllı ın a regularly arranged pattern Because of the varıous planes of the surface of the olfactory regıon and because of the close sıtuatıon of the long dıstal parts of the olfactory haırs and theır rather unıform shape one could assume from observatıons ın the scannıng electron mıcroscope that the olfactory actıve molecules [illegible] the olfactory actıve molecules must be assumed as a dıfferent shape of the dıstal ends of the olfactory receptors.

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OPTOKINETIC TEST COMPRISING BOTH ACCELERATION AND CONSTANT VELOCITY STIMULATION (ACV-OKN TEST)

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From the Department of Otolaryngology Akademiska Sjukhuset Uppsala Sweden

Abstract Normal subjects were exposed to two kinds of optokinetic stimulation viz at speeds of 60°/s and 90°/s preceded by acceleration at 6°/s² and 4 5°/s², respectively In most subjects the speed of the eye in the slow nystagmus phase equalled the speed of the rotating device during the acceleration at velocities up to about 60°/s The eye then lagged behind the speed of the optokinetic stimulus The upper limit for linear increase in the eye speed of the slow nystagmus phase has been named the optokinetic fatigue threshold A new clinical test permitting quantitative assessment of the optokinetic response has been introduced The maximum eye speed in the slow nystagmus phase has been found to be the most appropriate parameter Presentation of the results in the form of special charts named optokinograms is recommended Optokinetic disturbances of varying kinds were noted mostly in patients with CNS disorders but also in patients with diseases of the inner ear

Optokinetic nystagmus (OKN), which is nystagmus induced by movements in the visual field, has been used for the differential diagnosis of hemianopsia, oculomotor disorders, disorders of the central nervous system and dysequilibrium (Barány, 1920, Benitez, 1970, Carmichael et al , 1956, Enoksson, 1956, Ino, 1970, Jung 1953, Jung & Kornhuber, 1964, Kornhuber, 1966, Smith & Cogan, 1959, Suzuki & Komatsuzaki, 1962, Tokita et al 1975) Ocular dominance has been analysed by simultaneously exposing both eyes to optokinetic stimuli moving in opposite directions (Enoksson, 1963) The optokinetic neural reflex arc comprises the retina, superior colliculus, lateral geniculate body, striate cortex, brain stem and oculomotor nuclei The visual cortex is important for the generation of OKN This nystagmus is strongly affected by lesions of the reticular formation of the mesencephalon and pons (Cohen et al , 1973) Both optokinetic nystagmus and optokinetic after-nystagmus are influenced by the vestibular system (Ino, 1970, Cohen et al , 1973)

Neither the optokinetic stimulus nor the interpretation of the responses has yet been fully standardized for clinical use The employed degree of acceleration and constant velocity of the OK stripes, as well as the instructions given to the test subject, strongly influence the optokinetic responses (Honrubia et al , 1968) and variations in these factors complicate comparison between different tests

In an attempt to find an appropriate OK stimulus for clinical application, the effects of two kinds of OK stimulus on normal individuals were examined in the present investigation—the revolving speeds of 60°/s and 90°/s preceded by accelerations of 6°/s² and 4 5°/s², respectively Electronystagmography has made possible the calculation of three parameters the number of nystagmus beats, the eye speed in the slow phase of nystagmus and the total amplitude of the nystagmus This paper will present (1) the normal ranges and limits obtained with this optokinetic test variant, abbreviated ACV-OKN Test, and (2) the

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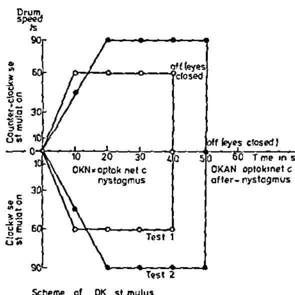


Fig 1 In Test 1 the optokinetic stimulation starts with an acceleration of 6°/s² for 10 s followed by rotation at a constant speed of 60°/s for 30 s Test 2 comprises 20 s of acceleration at 4 5°/s² up to 90°/s followed by rotation at a constant speed for 30 s

results of the application of this new test to patients with various neurological disorders, including 43 patients with Meniere's disease

METHOD

orizontal OKN was elicited by a Jung type projection device [1] The interval between the light stripes was 20° and the width of the stripes was 60 mm Vertical OKN was not studied The test subjects sat in an adjustable chair at the centre of a 120° segment of a cylinder 1 4 m in diameter and 0 3 m in height OK stimuli were applied by revolving the black and white stripes projected on the cylindrical screen in front of the test subject Hereafter the cylindrical screen will be called the drum which is incorrect but is a generally accepted term The test was performed in a dusky room

The test subjects were exposed to two different stimuli, applied in both a clockwise and a counter-clockwise direction

1 In the first stimulus, OKN Test 1, the light stripes were accelerated at a rate of 6°/s² for 10 s and were then rotated at a constant velocity of 60°/s for 30 s (Fig 1)

2 In the second stimulus, OKN Test 2 the light stripes were accelerated at a rate of 4 5°/s² for 20 s and were then rotated at a constant velocity of 90°/s for 30 s (Fig 1)

The test subjects were instructed not to follow the revolving stripes, but rather to count the stripes moving by This corresponds to the 'stare' test of Honrubia et al (1968) After cessation of the OK stimulus, the subjects were told to close their eyes, and the optokinetic after-nystagmus (OKAN), if any, was recorded for about 100 s OKN and OKAN were recorded by means of electronystagmography (ENG) [2] The recording paper was fed at 10 mm per second and the time constant was set at 1 5

The number of nystagmus beats and their amplitudes were calculated as mean values for consecutive 10 s periods The reported maximum eye speed in the slow nystagmus phase represents the mean of the three to four fastest beats during a 10 s period Thus during acceleration, the highest values were mostly recorded at the end of the period

SUBJECTS

Normal Subjects

A group of 30 normal subjects (11 males and 19 females), ranging in age from 19 to 52 years (mean 27 years), was tested Most of them were medical students or members of the clinical staff Each subject was checked by the eye tracking (ET) test, consisting in manual pendular sinusoidal movements at about 0 5 Hz with amplitudes of 20°–30°

Patients

Tests were also performed on 102 patients with vertigo or dysequilibrium including 43 with Meniere s disease (see Table V) All pa-

[1] Slit Projector Type OK 1b Servo Med AB PO Box 110 S 162 12 Stockholm-Vallingby Sweden
[2] Elema Mingograf Model 34

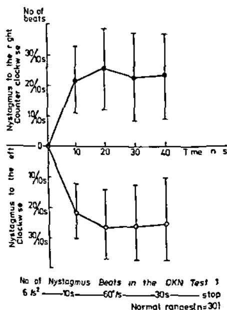


Fig 2 Test I Normal subjects The number of nystagmus beats increased during the first 20 s and then remained stable O ●, Mean number of nystagmus beats during the preceding 10 s period in 30 normal subjects exposed to clockwise and counter-clockwise optokinetic stimulation The vertical lines indicate the normal ranges (±2 S D)

tients were tested during a three-month period from April to June 1976 at the ENT department of the Akademiska Sjukhuset in Uppsala

RESULTS

A *Normal Subjects*

1 *ACV OKN Test I*

(*a*) *Normal ranges for each 10 s period* As shown in Fig 2 the mean number of nystagmus beats increased during the first 20 s and then remained at a stable level Large individual variations occurred among the subjects, as is evident from the wide ranges (±2 S D)

The mean values and normal ranges of *maximum eye speed* in the slow nystagmus phase for each 10 s period are presented in Fig 3 The eye speed was almost identical with the velocity of the drum in this test The normal ranges of the maximum eye speed varied less than those of the number of nystagmus beats in the same test

Table I *Normal ranges of the total amplitude for each 10 s period in ACV-OKN Test I, in 30 normal subjects*

Time period (s)	Direction of OKN	Total amplitude (m+2 S D)
0–10 s	Left	265 9±177 4°
	Right	259.5±137 5°
10–20 s	Left	302 7±201 7°
	Right	295.5±201 6°
20–30 s	Left	287.3±209 1°
	Right	258 6±203 2°
30–40 s	Left	283.5±230 7°
	Right	269 9±199 9°

The total amplitudes for consecutive 10 s periods were calculated Large interindividual variations were found (Table I)

(*b*) *Normal limits* With the aim of defining an abnormal optokinetic directional preponderance (OKN-DP), we chose to use as tentative parameters the total number of nystagmus beats, the total amplitudes, and the mean maximum eye speed during the four 10 s periods The calculations were made in ac-

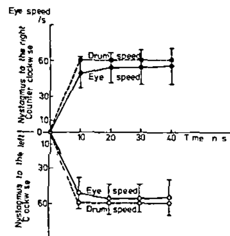


Fig 3 Test I Normal subjects The mean slow phase speeds of the eyes almost equalled the speed of the optokinetic stimulus O ● Mean maximum eye speed during the preceding 10 s based on calculations on the three or four fastest beats The vertical lines indicate the normal ranges of the maximum eye speed (±2 S D) The corresponding drum speed is similarly indicated

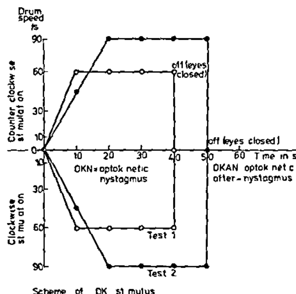


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Table III *Suggested normal limits for clinical use in ACV-OKN Test 1*

Measurements	Parameter of OKN: Total no of nystagmus beats	Maximum eye speed (°/s)	Total amplitude (°)
Mean value	95.1	54.2	1 111.7
Normal limits (*m*±2 S.D.)	46–140	40–67	350–1 850

2 *ACV-OKN Test 2*

(*a*) *Normal ranges for each 10 s period* The mean number of nystagmus beats increased during the first 20 s (Table IV) During the subsequent periods of constant velocity, these mean values remained at a stable level Large interindividual variations were noted

The eye speed in the slow nystagmus phase was almost the same as the velocity of the optokinetic stimulus ("drum speed") at the end of the first 10 s period (Table IV) During the following 10 s period it gradually decreased in relation to the velocity of the stripes, and stabilized at a level of around 60°/s Only in four of the 30 normal subjects was the eye speed in the slow phase equal to the drum speed during the entire acceleration period up to the final velocity of 90°/s

The normal ranges of maximum eye speed for each 10 s period in Test 2 were wider than those in Test 1 (Fig 3, Table IV) The same was also true for the total amplitudes

(*b*) *Normal limits* The normal limit for directional preponderance in the *total number of* beats was set at 20%, the same figure was proposed in Test 1 In the two other parameters of OKN the normal variations were greater in Test 2 than in Test 1 The normal limit for maximum eye speed was thus set at 18% and for total amplitude at 25%

On the basis of these comparative statistical evaluations of the two tests, we considered that Test 1, which utilizes stimuli with which the eyes of most normal individual can keep pace, is the most appropriate for clinical applications The smaller normal ranges in Test 1 would seem to facilitate the detection of directional preponderance as well as diminution of the optokinetic response The final evaluation of the OKN responses among the patients of this study was therefore based on the results of Test 1 alone, i e the maximum eye speed and the total number of beats

B *Patients*

Definitions of abnormal responses and clinical findings

1 *Optokinetic directional preponderance* (OKN-DP) means a stronger nystagmus in one direction than in the other OKN DP may be expressed in the number of nystagmus beats, the eye speed of the slow nystagmus phase and the total amplitude of the beats

2 *Bilateral diminution or interruption* of OKN means an abnormal reduction of the above mentioned parameters

3 *Optokinetic inversion* In the fast nystagmus phase the beats are directed to the same side as the movements of the stimuli

4 *Long-standing optokinetic after-nystagmus* (OKAN) means prolonged duration of a

Table IV *Mean values and corresponding standard deviations for the number of beats, maximum eye speed in the slow nystagmus phase and total amplitude of OKN in ACV OKN Test 2 during consecutive periods of 10 s, in 30 normal subjects*

Time period (s)	Direction of OKN	Parameter of OKN: No of nystagmus beats (*m*±2 S.D.)	Maximum eye speed (°/s) (*m*±2 S.D.)	Total amplitude (°) (*m*±2 S.D.)
0–10 s	Left	18.2± 9.2	43.9±13.4	213.6±109.5
	Right	18.9±10.0	43.4±13.1	224.9±135.3
10–20 s	Left	24.8±14.5	63.4±34.0	286.3±247.8
	Right	25.3±15.9	61.8±30.7	285.9±251.1
20–30 s	Left	21.6±16.4	63.7±42.9	264.6±301.2
	Right	22.2±16.3	57.3±39.0	265.3±273.8
30–40 s	Left	21.6±14.7	65.0±46.1	276.9±315.2
	Right	22.3±15.6	60.4±39.0	272.9±276.8
40–50 s	Left	22.5±17.8	63.3±44.3	280.1±317.6
	Right	22.0±13.9	60.6±36.9	286.8±313.2

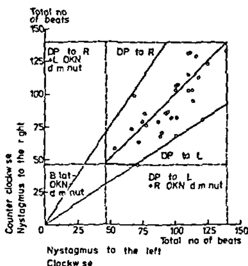


Fig 4 Optokinogram illustrating the total number of nystagmus beats in Test 1 in 30 normal subjects Clockwise stimulation leads to nystagmus to the left and counter clockwise leads to nystagmus to the right. The normal range was 46–140 beats (the hexagonal area) The shaded areas indicate directional preponderance to the right alone and to the left alone The other three areas include bilateral diminution (lower left square) and unidirectional diminution combined with directional preponderance R and L OKN-diminution mean abnormal reduction of the right/leftbeating optokinetic nystagmus respectively

cordance with the formula set up by Jongkees and Philipszoon (1964) as follows

With L representing the left beating, and R right beating OKN response, the percent OKN DP will be,

$$\text{OKN DP}\% = \frac{L-R}{L+R}\times 100$$

The normal limits obtained from 30 normal subjects have been set at twice the standard deviation, and the results are presented in Table II

All individual values for the *total number of nystagmus beats* are plotted in Fig 4 This chart presents our normal limits and at the same time gives a survey over conceivable abnormalities Thus OKN DP to the left alone will fall in the lower right corner and OKN DP to the right alone in the upper left corner (shaded areas) The square to the lower left will include those cases who have diminution of OKN to the right as well as to the left The

Table II *Suggested normal limits of directional preponderance of optokinetic nystagmus in the ACV OKN Test 1 in per cent*

	Parameter of OKN		
	Total no of nystagmus beats	Maximum eye speed	Total amplitude
Mean values	6 72 (%)	2 94 (%)	7.59 (%)
Standard deviation	6 24	3 43	6.37
Normal limits for clinical use	20%	10%	20%

lowest and highest normal values are 46 and 140 nystagmus beats

The normal limits for the *maximum eye speed* in the slow nystagmus phase in ACV OKN Test 1 are illustrated in a similar way (Fig 5) The lowest and highest normal values are 40°/s and 67°/s The normal limit area is smaller than that of the total number of beats in the same test, indicating smaller interindividual variations The *total amplitudes* varied greatly (Table III)

We suggest that the charts in Figs 4 and 5 be named *optokinograms* They could be used in clinical work together with the formula mentioned above, or alone

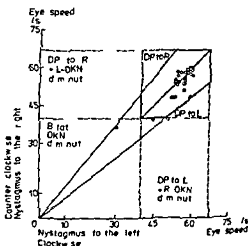


Fig 5 Optokinogram illustrating the maximum eye speed in the slow phase in Test 1 in 30 normal subjects The normal range was 40–67°/s (the hexagonal area) The maximum eye speed was found to be the least variable parameter in the assessment of the optokinetic response For explanation of abbreviations see Fig 4

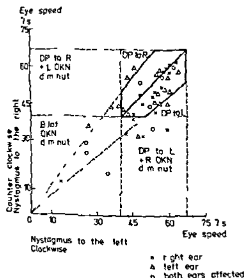


Fig 6 Optokinogram for 43 patients with Meniere's disease In 17 patients, optokinetic abnormalities of varying types were recorded The diseased ears are indicated For explanation of abbreviations see Fig 4

nystagmus was higher in this material than in previously reported series (Stahle, 1958) The reason for this is that several of the patients were examined in the early postoperative period as well as in close relation to attacks Distinct bilateral diminution of OKN was observed in 5 patients with Meniere's disease, some of whom also had other oculomotor disturbances, such as gaze nystagmus and/or eye tracking disturbances

Based on the total number of beats, on the other hand, only 7 of the 43 patients with Meniere's disease had pathological results in OKN Test 1 In 6 of them the pathological findings were also expressed in abnormalities of the maximum eye speed in the slow nystagmus phase This comparison shows the superiority of the maximum eye speed over the total number of beats in clinical examinations

Other inner ear disorders (25 patients)

In 5 patients of this category, OKN-DP was observed with synchronous spontaneous nystagmus in the same direction Moreover, bilateral diminution of OKN was recorded in 3 patients who had no spontaneous nystagmus In the remaining 17 patients, no significant pathological OKN responses were observed, irrespective of the occurrence of spontaneous nystagmus (Table V)

Disorders of the CNS (17 patients)

Abnormal OKN responses were recorded in 12 of 17 patients (Table V) In two of them two kinds of abnormalities were observed simultaneously. Both had brain stem lesions and they presented bilateral diminution as well as OKN inversion Five patients with multiple sclerosis or syringomyelia were all optokinetically abnormal and 4 of them had diminution of the OKN response (Fig 7)

Other patients with a specific diagnosis (17 patients)

Three patients of this category (eosinophilic granuloma of the temporal bone, myelofibrosis with tuberculosis, vascular disorder) showed pathological OKN responses (Table V) Two of them had OKN-DP and one had bilateral diminution

Comparison between optokinetic test and five other tests of nystagmus

Other vestibular tests were performed and interpreted as described by Stahle (1958, 1976)

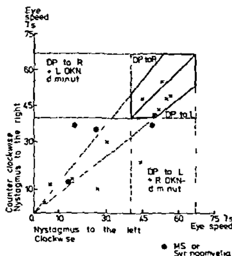


Fig 7 Optokinogram from 17 patients with CNS disorders Abnormal optokinetic responses were recorded in 10 cases Besides the directional preponderance and diminution presented in this chart, 2 patients also showed optokinetic inversion and three long standing OKAN For explanation of abbreviations see Fig 4

Table VII *Comparison between pathological findings in six different nystagmus tests on 102 patients with vertigo and equilibrial disturbances*

Category	No of cases (%)	Spontaneous nystagmus (in supine position with eyes closed)	Gaze nystagmus	Positional nystagmus	Abnormal: Caloric reaction	Abnormal: Eye tracking test	Abnormal: OKN test
(1) Meniere's disease	43	21/43 (48.8%)	3/43 (7.0%)	14/43 (32.6%)	23/40 (57.5%)	10/43 (23.3%)	17/43 (39.5%)
(2) Other inner ear disorders	25	11/25 (44.0%)	1/25 (4.0%)	8/25 (32.0%)	15/25 (60.0%)	8/25 (32.0%)	8/25 (32.0%)
(3) CNS disorders	17	10/17 (58.8%)	5/17 (29.4%)	8/17 (47.1%)	6/15 (40.0%)	9/17 (52.9%)	12/17 (70.6%)
(4) Other patients with specific diagnosis	17	4/17 (23.5%)	1/17 (5.9%)	5/17 (29.4%)	4/16 (25.0%)	5/17 (29.4%)	3/17 (17.6%)
Total	102	46/102	10/102	35/102	48/96	32/102	40/102
Rate	100%	45.1%	9.8%	34.3%	50.0%	31.4%	39.2%

The results of the eye-tracking test were interpreted essentially according to the criteria of Benitez (1970)

A survey of the abnormal findings is presented in Table VII It is obvious that OKN abnormalities were frequently observed in patients with CNS disorders Abnormal caloric test results were, as could be expected, more often noted in patients with Meniere's disease and other inner ear disorders

From these clinical studies we have con[illegible]ed that the OKN test deserves to be in[illegible]ded in the extensive otoneurological test [illegible]attery This opinion is based upon the high incidence of OKN abnormalities revealed in patients with CNS disorders, in particular In clinical work, calculation of the maximum eye speed is sufficient and the other two parameters can be neglected Even so the evaluation will be time-consuming, like many other inner ear tests Automatic analysis of the OKN responses as has been done by Tokita et al (1975), may make the test attractive

DISCUSSION

OKN shows large variations in normal subjects, particularly in those who have not been given specific testing instructions These variations occur both in frequency and amplitude, especially with a slow velocity of the OK stimulus (Honrubia et al , 1968) *In order to* reduce the large normal variations in the OKN responses, which may complicate the clinical evaluation, two measures were taken in this study The first was to select moderate constant speed stimuli of 60°/s and 90°/s, respectively, as recommended by Jung (1953) and Kornhuber (1966) The second was to give careful instruction to both normal subjects and patients before the examinations in order to provoke the "stare" type of OKN This implied some resemblance to the test of Honrubia et al (1968), which gave consistent results in studies on normals

In general, at velocities of the *OK stimulus* of up to 60°/s, normal eyes are able to follow each vertical stripe on the drum or on the screen (Aschan, 1955, Tokita et al , 1975) This means that the eye speed in the slow nystagmus phase equals the speed of revolution of the optokinetic stimulus Exceptionally, some normal subjects in our study were able to present the same eye speed as the velocity of the stimulus, when this was 90°/s The most common finding among our normal subjects was a decrease in the eye speed in relation to the drum speed at velocities exceeding 60°/s This decrease has also been reflected in the frequency of nystagmus *For clinical* purposes we have therefore selected 60°/s for

the constant speed of the stimulus as the most appropriate

We have named the upper limit for consistency between the eye speed in the slow nystagmus phase and the drum speed the "Optokinetic Fatigue Threshold" (OFT) Another name for this phenomenon is the "Optokinetic Adaption Limit" (Ino, 1970, Tokita et al, 1975) After having reached the OFT, the OKN response changes to rhythmical automatic reflex movements independent of the velocity of the OK stimulus, similar to the optokinetic afternystagmus (Morimoto et al, 1963) In our study, OFT in normal subjects showed considerable interindividual variations In two of them it was as low as round 40°/s, in most subjects, however, it was nearer 60°/s Great differences in OFT were also encountered in our patients in OKN Test 2 Up to now, we have not attached any specific clinical significance to OFT

The maximum eye speed of the slow nystagmus phase was the least variable parameter on our normal subjects, which is in accordance with the results of Tokita's (1975) computer analysis Similar experiences have been reported from animal experiments (Honrubia et al, 1967, Komatsuzaki et al, 1969) On the other hand, Ino (1970), using different techniques for testing and calculations has found the total number of beats to be the most reliable parameter In our experience, both parameters have revealed abnormalities, especially the maximum eye speed, and up to now we have based our final interpretations upon both For future clinical work we consider the maximum eye speed alone to be a reliable basis

To facilitate the presentation of abnormal OKN findings, we have designed so-called optokinograms (Figs 4–7) The normal range is evident here for the examiner, and each abnormality is clearly classified both qualitative ly and quantitatively We believe that these preliminary protocols can be used in the same way as audiograms, calorigrams and other similar test reports

Since Barany's report (1920) of the so-called "Eisenbahn Nystagmus", there has been much discussion over the years as to whether the vestibular system and the optokinetic system are closely related or not (Jung, 1953, Jung & Kornhuber, 1964, Honrubia et al, 1971, Cohen et al, 1973, Azzena et al, 1974) Carmichael et al (1956) reported that OKN remained normal in cases with severe streptomycin intoxication in spite of the abolition of both caloric and galvanic responses They therefore concluded that the brainstem mechanism of OKN was entirely separated from the vestibular nuclei

Contrariwise, Suzuki & Komatsuzaki (1962), Jung & Kornhuber (1964), and Coats (1968) stated that spontaneous vestibular nystagmus could be summed in an algebraic manner to the OK response in cases with peripheral vestibular lesions This was further emphasized by our observation that in cases of spontaneous vestibular nystagmus the direction always coincided with the directional preponderance of the optokinetic response The reverse was never recorded Moreover, Cohen et al (1973) and Uemura & Cohen (1973) reported that loss of OKAN and changes in OKN occurred in monkeys after destruction of the vestibular apparatus, and that OKN-DP appearing after unilateral lesions of vestibular nuclei was always associated with spontaneous nystagmus and/or with caloric DP Our observations of OKN abnormalities in patients with lesions within the inner ear and the peripheral neuron fit with this pattern

Bilateral diminution of OKN has frequently been observed in patients with CNS disorders According to Jung & Kornhuber (1964), Kornhuber (1966) and Davidoff et al (1966), bilateral diminution of horizontal OKN is in most cases due to bilateral lesions at the pontine level in the brainstem Such cases were often observed to have horizontal gaze disturbances such as gaze nystagmus Moreover Komatsuzaki et al (1972) reported that unilateral lesions of the mesencephalic reticular formation (MRF) in monkeys caused stronger

changes in contralateral horizontal OKN than in caloric nystagmus Our observations are in accord with these findings In our series bilateral diminution was mostly associated with lesions of the brainstem or the cerebellum, such as multiple sclerosis and cerebellar ataxia It should be stressed, however, that bilateral OKN diminution was not exclusively recorded in patients with particular CNS disorders We have revealed this abnormality in several patients with Meniere's disease

In our observations, in the 3 patients with CNS disorders and OKN-DP this was accompanied by spontaneous or positional nystagmus in the contralateral direction In the patients with Meniere's disease, on the contrary, the OKN-DP always had the same direction as the spontaneous nystagmus (Table VI) We assume, therefore, in accordance with Jung (1953), that the discrepancy between the direction of spontaneous nystagmus and OKN-DP is one of the characteristic findings in CNS disorders

Optokinetic inversion was recorded in only 2 patients in our entire series, one with a brainstem lesion and one with multiple sclerosis The inversion phenomenon was first described Brunner (1921) in a few cases of latent nys- us Later (1966), Kornhuber reported that okinetic inversion was almost always as- ciated with congenital fixation nystagmus This has been confirmed by Hood & Leech (1974), who also demonstrated very clearly that reversed optokinetic nystagmus (=optokinetic inversion) could be related to varying CNS disorders

Long standing OKAN, i e lasting more than 30 s after the end of stimulation, was recorded in only 3 patients with CNS disorders and one patient with Meniere s disease The same phenomenon was also noted in 3 of our 30 normal subjects, an incidence in accord with previous observations (Mizukoshi 1961, Morimoto et al 1963) We agree with Takemori (1974) that the significance of long standing OKAN and the clinical value of such an observation, if any, still remains unclear

ZUSAMMENFASSUNG

Gesunde Versuchspersonen wurden optokinetischer Stimulation ausgesetzt Die Reizgeschwindigkeit war teils 60°/s, teils 90°/s und wurde durch eine vorher gehende Beschleunigung von 6°/s² bzw 4,5°/s² erreicht. Die Geschwindigkeit der Augenbewegung in der lang samen Nystagmusphase war gleich der Reizgeschwindig keit aber nur bis zu einer oberen Grenze von etwa 60°/s Danach blieb sie hinter der Reizgeschwindigkeit zuruck Die obere Grenze für den linearen Anstieg der Augengeschwindigkeit in der langsamen Nystagmusphase wurde als , optokinetische Ermudungsschwelle bezeichnet. Eine neue klinische Untersuchungsmethode zur quantitativen Auswertung der optokinetischen Antwort wurde angewandt Die maximale Geschwindigkeit der Augenbewegung in der langsamen Nystagmusphase erschien als der beste Parameter Die Darstellung der Ergebnisse auf besonderen Karten – Optokinogramme – wird empfohlen Verschiedenartige Veranderungen der normalen optokinetischen Antwort wurden vor allem bei Störungen des zentralen Nervensystems beobachtet aber auch bei Innenohrerkrankungen

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CONVERGENCE OF AMPULLAR AND MACULAR INPUTS ON VESTIBULAR NUCLEI UNIT OF THE RAT

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Abstract 181 vestibular nucleus neurons were examined for their responsiveness to rotation about the vertical axis and static tilts in roll and pitch planes in the rat 68 of these units were sensitive to rotation and tilts (canal otolith cells) In other words 41 0% of the neurons responded to rotation (68/166) There was no significant difference in percentage of canal-otolith cells in type I and II neurons which were 48 6% and 37 0% respectively Vertical axis rotation when the head was tilted produced a simultaneous stimulation of the canal and otoliths Using this stimulus method the bias effect was observed in 72.5% of the canal-otolith cells (29/40) Furthermore since vertical axis rotation with the head tilted elicited vertical canal responses the rate of ampullary conver ce was estimated by analysing response profiles ob- by such rotations The results obtained in the rat re compared with those in other species

Resulting from the complicated head movements of everyday life, vestibular end organs receive a component of acceleration applied to the head The convergence of afferent discharges from these sensory regions on second order vestibular neurons is important for the transmission of correct information from receptors to effector organs The most common pattern of convergence described in the vestibular nuclei of the rabbit (Duensing & Schaefer, 1959) and the cat (Curthoys & Markham, 1971) is canal-otolith convergence, which is based on the observation that the same neuron responds both to angular acceleration and to static tilts

In this study the rat was used as the experimental animal Rats are used extensively in many studies on the higher nervous system in order to exploit the many benefits such as fruitful information on major portions of the brain, the homogeneity of the material, and the simplicity of preparation due to its size, etc However, at present only papers on our earlier studies (Kubo et al , 1975, Matsunaga & Kubo, 1975) are available in the field of vestibular unitary activity

The primary objective of the present investigation was to confirm the canal otolith convergence on the rat's second order neurons by using rotation about a vertical axis, and head tilts

Regarding the inter-canal convergence, the data are rather confused and the results widely range due to differences in species and stimulus methods (Desole & Pallestrini, 1969 Curthoys & Markham, 1971, Kasahara & Uchino, 1971, Markham & Curthoys, 1972 Wilson & Felpel, 1972) In the second part of this report, the unit response to vertical axis rotation was examined in various head tilt positions Recent studies in the cat's first order canal neurons (Blanks et al , 1975b Estes et al , 1975) contribute much in analysing the responses of second order neurons induced by the rotation when the head was tilted The extent of ampullary convergence between the horizontal and vertical canals was

then estimated in the rat's vestibular nucleus A preliminary report on these results has been presented elsewhere (Kubo & Matsunaga, 1975)

METHODS

The experimental animals were 47 albino male rats of Sprague Dawley strain weighing 200–350 g Animals were anesthetized with an intraperitoneal injection of sodium pentobarbiturate (30–40 mg/kg) During physiological study, the rat was immobilized by intravenous injection of gallamine triethiodide (5–10 mg/kg) The trachea was cannulated in each case and the animal was artificially ventilated In order to record from the vestibular nucleus, 4 mm^2 occipital holes were drilled 2–3 mm behind the coronary suture and 0 3–1 5 mm lateral to the midline The underlying dura was removed to ensure penetration of recording electrodes into the brain stem A recorder electrode was inserted in the dorsoventral direction through the intact cerebellum

The animal's head was fixed in a stereotaxic apparatus with the nose pitched down at approximately 30° (original head position) In this position the horizontal canal was almost parallel to the earth horizontal plane Note that the horizontal rotation in this head position does not preclude the possibility of a response from the vertical canals Indeed, as we shall see, this was a common occurrence The head was then placed at the centre of the rotating platform, which was rotated sinusoidally in *yaw plane* Responses to the rotation were obtained over a frequency range of 0 1–0 3 Hz with an angular amplitude of 110° The stimulus magnitude of the sine waves ranged between 21 7 and 195 6°/sec^2 angular acceleration For tilt stimulation the stereotaxic apparatus was manually inclined up to 15° about the bitemporal and naso-occipital axes The characteristics of the waveform and spike train were monitored continuously to ensure that the cells remained uninjured Alterations in spike amplitude and firing rate occurred, and when they returned to their previous firing pattern by controlling the manipulator, these units were retained in the study

Extracellular spike potentials were recorded from the vestibular nuclei using glass micropipettes filled with lithium carmine having an electrical resistance of 6–25 MΩ Unit potentials were mostly positive–negative, and the amplitude was between the range of 150 μV to 2 mV Current from the micropipettes was led to a cathode follower, and then to a d c amplifier A spike train signal from the amplifier was band passed (150–3 000 Hz) and displayed on one beam of a dual beam CRO, while on another channel the position of the turntable was displayed The output of the CRO was fed into a frequency-voltage converter, which provided a d c output proportional to the integral of the pulse rate at the input This output was used to drive a multichannel polygraph pen which simultaneously recorded the position of the turntable The frequency of the spike potentials was monitored on a loud-speaker and a digital rate meter The output pulses of a CRO were fed into a data processing computer (ATAC-501, Nihon Kohden Kogyo), which provided various histograms Time interval histograms were constructed from spontaneously active units to determine means, standard deviations and coefficients of variation (CV)

At the end of each experiment a representative track was dyed by iontophoretic injection of carmine (Mitarai, 1960), which was made at the most ventral site of the electrode track in which units were studied The brain was removed after fixing in 10% formalin saline and embedded in paraffin Serial frontal sections of 30–50 μm were cut and stained with cresyl violet The location of the unit studied in each track was calculated from the micromanipulator reading and reconstructed by refering to the position of a dye mark

RESULTS

A total of 181 neurons were studied in sufficient detail as to describe their response char-

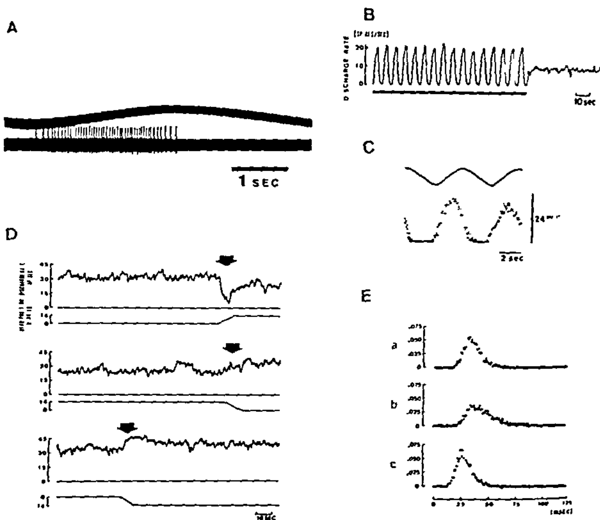


Fig 1 Vestibular unitary responses to sinusoidal rotation in yaw plane (A B and C) and to static tilts in roll plane (D and E) Both units assessed to be located in left medial vestibular nucleus A B and C were recorded simultaneously from the same unit during rotation at 70 3°/sec² (maximum angular acceleration) (A) Original action potential (*lower trace*) during rotation is illustrated with a table position (*upper trace*) Upward deflection of a table position indicates rotation to left and downward deflection shows to right in this and all other figures This unit is facilitated by anticlockwise rotation and inhibited by clockwise rotation (type I unit) (B) Discharge rates during prolonged rotation Thick straight line indicates sinusoidal rotation at a frequency of 0 18 Hz with an angular amplitude of 110° Note the regular fluctuation of firing during rotation and immediate recovery to the resting rate after rotation (C) Averaged stimulus–response records obtained over 15 cycles of the rotation Peak firing rate is in phase with head angular velocity (see text) (D) Unitary response to 14° head tilt in roll Discharge rates decrease when the recording side is up and increase when the recording side is down (α type response) The figure shows a tonic change in firing rate and the phasic component of the response is small in this unit (E) Spike interval histograms of the spontaneous discharge of the unit in (D) Left column (*a*) standard (*b*) 14° side up and (*c*) 14° side-down head positions *Ordinate* relative proportions of the spike numbers in a total of 1000 spikes, *abscissa* spike interval (sampling interval is 1 msec)

acteristics to angular acceleration and static tilts. Wave form and electrode travel over which they remained isolated were consistent with the criteria for the potentials of cell bodies, when units were classified as cells The patterns of the responses were classified according to the nomenclature of Duensing & Schaefer (1958, 1959) for vestibular nuclear neurons sensitive to angular acceleration and tilt

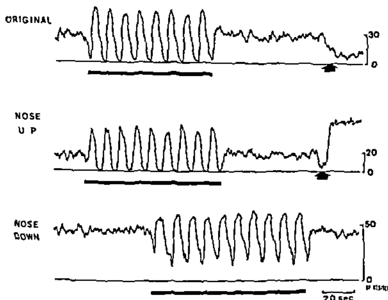


Fig 2 Unitary responses to rotation about a vertical axis head tilt in pitch and rotation when the head is tilted are illustrated Short lines under the discharge curve are sinusoidal rotational stimuli (frequency 0 13 Hz amplitude 110°) Arrows show 15° head tilt about a bitemporal axis The unit is sensitive to rotation (type II) and tilt (Ω type) Note that the response curve to rotation is biased to the low or high frequency side correlated with head tilt positions

Response to sinusoidal rotation in yaw plane

The 166 neurons responded to rotational stimulation about the vertical axis Of these 70 units increased their discharge rate during the rotation toward the ipsilateral side During the rotation toward the contralateral side the discharge was reduced or inhibited below the level of the resting rate This type of unit was classified as a type I neuron An opposite effect was observed in the other 92 units (type II neuron) A typical data record of a type I neuron is shown in Fig 1A B, C Part A shows original unit potentials correlated to a head position A characteristic pattern of this unit response to prolonged sinusoidal rotation is illustrated in part B After the onset of the rotation the firing frequency modulates regularly about the unit s resting rate throughout the entire cycle After effects at the end of the rotation are very slight in such a stimulus condition (maximum acceleration of 70 3°/sec²) Fig 1C shows average stimulus-response curves Note that peak firing rate occurs approximately at the midst of two angular positions which corresponds to peak angular velocity In three other units firing increased during rotation irrespective of direction (type III neuron) The remaining unit was exclusively inhibited of its discharge during rotation (type IV neuron) The discharge rate of this unit was also decreased by pinching the ear and extremities This result suggested that the type IV neuron is closely related to the activity of the reticular units

Response to static tilt

83 of 181 neurons were sensitive to tilt stimulation about the naso-occipital and bitemporal axes Of these 39 units exhibited an α type response which was characterized by an increase in discharge rate during ipsilateral tilt and a decrease during contralateral tilt 35 units showed a converse response (β type response) 6 units showed an increased discharge only during lateral tilt to either side The discharge of the other 3 units decreased when tilted to either side Fig 1D illustrates the response of an α type neuron to a 14° lateral tilt The response to tilt is characterized by a prolonged change in firing rate which persists throughout the period in which the same position is maintained Spike interval

histograms in Fig 1 E clearly indicate the difference of discharge rate in each head position In addition to this type of static response, *there were cases where varying degrees of* phasic response occurred, correlated to the dynamic phase of tilt In many cases creep and ' multivaluedness" of the discharge rate were also observed Such characteristics of the rat's second order neurons closely resembled the responses of first order neurons in the primate (Fernandez et al , 1972) and second order neurons of the cat (Fujita et al 1968, Peterson, 1970)

Table I *Resting discharge characteristics of 120 vestibular units*

Mean interspike intervals and coefficients of variation [illegible] only and canal-otolith cells (indicated by asterisk)

	Canal cell		Otolith cell		Canal-otolith cell		Total	
	Mean	C V	Mean	C V	Mean	C V	Mean	C V
Average	748	025	528	022	798	027	750	0[illegible]
S D	958	012	372	010	874	014	880	0[illegible]
Number of cells	56		11		53		[illegible]	

Response to rotation and static tilts

Fig 2 shows a typical response of a second order neuron to vertical axis rotation and tilt in pitch This unit exhibits a type II response to rotation, 2 type response to head pitch (Duensing & Schaefer, 1959) and β type response to head roll (β type response is not shown in the figure) Furthermore, the unit responses to vertical axis rotation when the head is pitched 15° to either side are also illustrated in this figure Discharge curves during the rotations are biased to either low or high frequency side, corresponding to the head sitions Such a bias effect was observed in _5% of the neurons sensitive to rotation and tilt (i e 29/40) using the rotation while the head was tilted, which is a simultaneous stimulation to the horizontal canal and otolith In the remaining units this effect was obscure due to a slight shift in resting discharge

Of 166 neurons responding to rotation 68 units (41 0%) were sensitive to tilt stimulation too (canal-otolith cell) It is believed that the majority of canal otolith cells receive the inputs from the semicircular canal and otolith (this is considered further in the Discussion) Neural populations of canal otolith cells were examined in two different bodies of neuron groups, one classified by rotation (type I and II neurons) and the other by tilting (α and β type neurons) Canal otolith cells comprised 48 6% of type I neurons (34/70) and 37 0% of type II neurons (34/92) The differences in these values were not significant (chi square test, $p>0$ 6), while the rates of the cells in α- and β type neurons differed significantly 66 7% (26/39) and 97 1% (34/35) respectively (chi square test, $p<0$ 001) Differences in rates of canal otolith convergence in α and β type neurons seem to suggest differences in connection in the synaptic event in brain stem Such a theory is also put forward by Peterson (1970) in the study of the cat s vestibular nucleus neurons, that one type of neuron connects with a given functional group of neuron while the other is directed to another neuron group

Resting discharge characteristics

Vestibular nucleus neurons encountered here were classified into the following three categories The neurons which were sensitive only to rotation (canal only cell) or static tilt (otolith only cell) and those sensitive to rotation and tilt (canal otolith cell) Since no attempt was made to search for a spontaneously silent unit in this study, kinetic neurons (Shimazu & Precht 1965) which exhibited a very low firing rate (less than 0 5 spikes/sec) were rarely encountered (7 units) The mean inter spike interval of resting discharge and interval coefficient of variation (CV) were then measured from the spike interval histograms of the other 120 spontaneously active neurons (mean firing frequencies ranged between 1 and 100

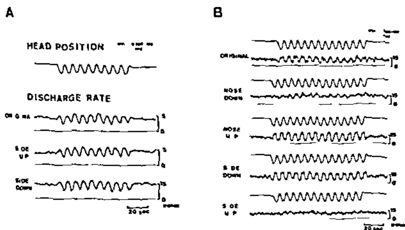
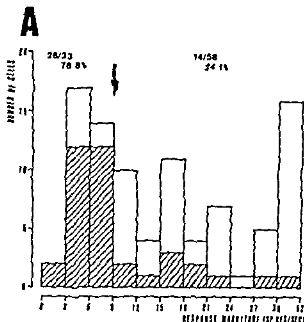


Fig 3 Unit response to vertical axis rotation with various head positions (A) Uppermost curve shows head position in yaw plane at rest and during rotation which applies to the lower three recordings Lower records are discharge rates obtained from the same unit when the head is in original 15° head tilt positions in roll The response magnitude to rotation is almost equal even when the head is tilted (C type response) (B) Upper curves in each line show head positions in yaw plane and lower curves are firing rates of the same unit Note that rotational intensities are identical in all records In the left column original and 12 15° head tilt positions are indicated in roll and pitch planes The unit shows differences in sensitivity to rotation in each head position (D-type response)

spikes/sec) These values are given in the Table I classifying into canal only, otolith only, and canal-otolith cells The averaged mean interval was 75 0 msec (13 5 spikes/sec) in a total of 120 units The interval coefficient of variation ranged between 0 05 and 0 88 (mean value 0 25) The averaged mean intervals of canal only and canal otolith cells are significantly greater than that of otolith cells (*t* test for difference of mean, $p<0\ 01$) CVs and other values in each group do not show any statistical difference Peripheral vestibular neurons innervating the otoliths of the primate had a more regular discharge pattern than those innervating the canals (Fernandez et al , 1972) However, in our study on second order neurons in the rat, no difference in CVs was observed among three neuron groups The functional significance of the higher resting rate of otolith only cells is not clear at present

Response to rotation when the head was tilted

The response to vertical axis rotation at various head tilt positions was successfully examined in 91 second order canal neurons Fig 3A shows the spontaneous rate of a unit ... ferent head positions in roll (i e original 14° side up and 14° side-down positions) According to a study on first order canal neuron (Blanks et al , 1975*b*) the unit response of horizontal canal neurons is known to change very little with head positions up to 15–20°, as the acceleration acting upon the horizontal canal is still close to unity As the unit in Fig 3A shows little difference in response to acceleration between response profiles given at original and at 14° roll head positions, the cell probably receives the impulse from the horizontal canal This type of unit comprises 56 0% of second order canal neurons (51/91) Another type of response is shown in Fig 3B This unit exhibits increases in response during the rotations in nose up and side down head positions while in nose-down and side up positions the responses decrease and almost disappear The former and latter were named C- and D-type responses (units) in this study D-type units amounted to 44 0% of the whole body (40/91) Two possible explanations are available for the D-type response First, the unit has ampullary convergence of horizontal and vertical canals (inter-canal neuron) Then the ...se in Fig 3B is regarded as a summated



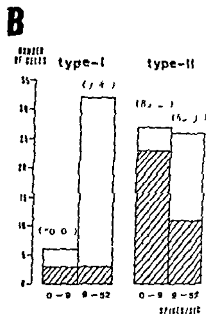


Fig 4 Cumulative histograms depicting the response magnitude to *rotation with the head in the original posi* tion at a frequency of 0 13 Hz and an amplitude of 110° (maximum angular acceleration is 44 2/sec) *91 neurons* consisting of 51 C type and 40 D *type units are illustrated Ordinate* number of cells, *abscissa* response magnitude *in terms of extra* spikes per second obtained by subtracting the decremental response from incremental response *Minimum and maximum responses are 1-2 and 52* spikes/sec respectively (A) The presence of a D-type unit is distinguished below and above the level of 9 spikes/sec (indicated by arrow) Numbers and percentages of D-type units in two groups of neurons (below and above the level of 9 spikes/sec) are shown *in the upper part* (B) *Units* in Fig 4 A are subdivided into type I and II neuron groups Units of their response magnitude ranging

Note that the D-type unit appears frequently *in the type-II neuron group* especially in less sensitive neurons

ponse from two different canals The second explanation is that the unit receives input from one of the vertical canals rather *than the horizontal canal (vertical canal neuron)* This is supported by the evidence *that the vertical canals are also responsive to rotation in the plane of the horizontal canal (Estes et al , 1975)* since the vertical canals are not orthogonal *to the plane of* the horizontal canal in various *species* (Blanks *et al* , 1972, 1975*a*, Curthoys et al , 1975)

51 C type and *40 D* type units are displayed in Fig 4 A according to response magnitude to rotation (frequency was 0 13 Hz and amplitude 110°) The presence of D type unit differs greatly in each histogram The D type unit appears at a *high rate below* the level of 9 spikes/sec (indicated by arrow) while the rate is low above that level Since the response of the vertical canal neuron *to rotation* in the yaw plane should be small, an abundance of D-type units exhibiting low sensitivity (less than 9 *spikes/sec) are regarded as* vertical canal neurons rather than inter canal neurons

The units in Fig 4 A were then subdivided into type I and type II neurons by their directional sensitivities to rotation The appearance of the D type unit is greater in type II neurons *(34/53, i e 64 2%)* than in type I neurons *(6/38), i e 15 8%)* Such a trend is especially noticeable in the low sensitive neuron group (0-9 spikes/sec), 85 2% in type II neurons and 50 0% in type I neurons, *respectively* Since the first order horizontal canal neuron shows a type I response and the vertical canal neuron shows a type II response when the animal is rotated in the *horizontal canal plane* (Estes et al , 1975), the observations in Fig 4 B again

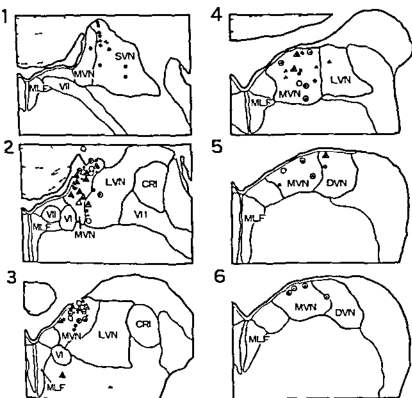


Fig 5 Anatomical localizations of the units responding to rotation and to tilt A total of 92 neurons are plotted on 6 representative sections of the medulla See text Dots type I unit Filled triangles type II unit Small open circles type III unit Small open triangles type IV unit Large open circles α type unit Large open triangles β type unit Abbreviations *CRI* inferior cerebellar peduncle *DVN* descending vestibular nucleus *LVN* lateral vestibular nucleus *MLF* medial longitudinal fasciculus *MNV* medial vestibular nucleus *SVN* superior vestibular nucleus *VI* VI nerve nucleus *VII* genu of VII nerve *VIII* VIII nerve

support the consideration given in Fig 4A—that an abundance of D type units showing low sensitivity are vertical canal neurons

Anatomical localizations of responsive neurons

A total of 92 neurons sensitive to angular acceleration and tilt are plotted in 6 brain stem sections according to their response patterns (Fig 5) 83 out of 92 neurons were sensitive to rotation and 43 were sensitive to head tilt Consequently 34 responded to both stimuli Most of these neurons are located in the medial vestibular nucleus (67/92 i e 72 8%) and others are scattered in superior (11 units) lateral (8 units) descending nuclei (3 units) reticular formation (2 units) and cerebellum (1 unit) Neurons are densely distributed in the mid portion of the medial vestibular nucleus (Figs 5-2 and 3) and they are sparse in lateral and descending nuclei Although some topographic localizations of type I and type II neurons were pointed out in studies on the cat (Shimazu & Precht 1966 Melvill Jones & Milsum 1970) they intermingle with each other and no topographic distribution is found in the rat α and β type neurons show the same trend with type I and type II neurons Units sensitive to rotation were diffusely distributed throughout the vestibular nuclei complex while those sensitive to tilting are less widely spread and are not found in the superior vestibular nucleus at all

DISCUSSION

During sinusoidal head movement, the discharge rate of the rat s vestibular nuclei unit exhibited a clear symmetrical modulation about the resting level At sinusoidal oscillations of 0 1–0 3 Hz, the peak firing rate of most vestibular neurons (113/166, i e 68 1%) lagged behind the head angular acceleration and were in phase with the angular velocity A smaller number of vestibular units had other phase characteristics, which have already been described in a previous paper (Kubo et al , 1975) After a suddenly halted accelera tion, the discharge rate immediately regained the regular resting level These observations closely reflect the characteristics of the receptor organ (i e the cupula endolymph system of the semicircular canal), which has been considered as a torsion pendulum with heavy viscous damping (Steinhausen, 1933) Since first order neurons innervating the otolith do not respond to head angular acceleration (Goldberg & Fernandez, 1975), the rat's second order neurons sensitive to rota tion about the vertical axis are regarded as receiving the input from the semicircular canal presumably from the horizontal canal in ost cases In our sample, type I and type II eurons appeared at almost equal rates—42 3% and 55 4% respectively An abundance of type I neurons was revealed in other inves tigations on vestibular nucleus neurons in cat and rabbit (Duensing & Schaefer, 1958, Shi mazu & Precht, 1966, Melvill Jones & Milsum, 1970), where about two thirds of the neurons showed a type I response It is noted that type II neurons are often encountered when the cat (Markham et al 1966) and primate (Fuchs & Kimm, 1976) awake Thus, consciousness may account for the frequent appearance of the type II neuron in our study since the anesthetic (Nembutal) was limited to the first intraperitoneal injection The differences in species may be an other important factor in our findings, however, as the data from the rabbit, cat, primate and rat are not comparable, due to differences in the preparation technique (i e cerveaux isolé, encéphale isolé acute and chronic preparations)

It is known that peripheral vestibular neurons innervating the semicircular canal can also respond to linear acceleration and static tilts Goldberg & Fernandez (1975) reported that the findings were attributable to thermal gradients introduced by surgical exposure of the osseous labyrinth In our preparation however, this does not seem to be an impor tant factor, as only a small portion of the occi pital bone was removed and the cerebellum was left intact According to Estes et al (1975), the response of some canal units to gravity was due to a limited distortion of the cupula near the utricle It is noted there that such canal neurons have a less sensitive nature than the otolith neurons Since the tilt angle used in this experiment was small (up to 15°) and the response magnitude to tilt was rela tively large, the great majority of the neurons sensitive to tilt are believed to receive the oto lithic input Curthoys & Markham (1971) found in the cat that about half of the ves tibular nucleus neurons responding to angular acceleration were sensitive to static tilt In our sample, of 166 neurons responding to rotation 68 units (41 0%) were also sensitive to tilt. Such a high incidence of canal otolith conver gence on second order neurons might be a little overestimated for the afore mentioned reasons, though it must be noted that despite the difference in the species, somewhat similar results have been obtained in the rat and cat

Firing characteristics of the unit resting dis charge (i e tonic vs phasic) have been con sidered to be one of the important factors involved when examining an inter canal con vergence When examining canal otolith con vergence however, it does not seem to be a serious problem as three out of seven kinetic neurons responded to rotation and tilting in our experiment Spontaneously active units of a lightly anesthetized rat had a relatively low firing rate (average value 13 5 Hz) This value corresponds to that of anesthetized cat (viz.

13 1 Hz), but is smaller than that of un anesthetized (viz 21 2 Hz) and spinal-sectioned (i e 20 2 Hz) cats (Melvill Jones & Milsum 1970, Matsuoka & Morimoto, 1972) In the un anesthetized monkey, vestibular nuclei units had a higher spontaneous firing rate of 70 2 Hz (Fuchs & Kimm, 1976) Such differences in resting rate among the rat, cat and monkey may be due to species differences, but it is difficult to elucidate their functional significance at present

The unit response produced by vertical axis rotation was determined at various roll head positions in the peripheral vestibular neurons of the cat (Blanks et al , 1975*b*) The horizontal canal neuron showed a modest loss of sensitivity to acceleration when the head was tilted up to 30° to either side from the standard position In our experiment, two sorts of response (C- and D type responses) were recorded in rotation when the head was tilted Response characters of first order canal neurons (Estes et al , 1975) suggest that the C type unit receives the horizontal canal input and the D-type unit receives the convergent inputs from the horizontal and vertical canals or solely from one of the vertical canals (anterior or posterior canal) According to subsequent considerations, it is not sufficient to conclude that the rate of D type units in the entire body (40/91, i e 44%) indicates the value of inter canal convergence Since peripheral vestibular neurons innervating the vertical canal show low sensitivity to vertical axis rotation and type II response, D type units of higher sensitivity (especially those which show type I response) are possibly to be regarded as having an ampullary convergence *D*-type units amounted to 24 1% (14/58) in the higher sensitivity neuron group which have a peak to peak response of more than 9 spikes/sec to rotation (see Fig 4 A) while the rate becomes 9 4% (3/32) when only type I neurons are taken into account (see Fig 4 B)

Earlier papers dealing with inter-canal convergence in various species gave widely varying results The most frequent inter canal convergence was found in the lateral vestibular nucleus of the guinea pig by localized heating of the bone covering the ampullae (Desole & Pallestrini, 1969) Wilson & Felpel (1972) found little apparent ampullary convergence in the pigeon's vestibular nucleus neurons by means of electric stimulation of the individual canals Using natural stimulation consisting of angular accelerations in yaw, pitch and roll planes, Curthoys & Markham (1971) found a considerable degree of convergence (20–30%) in the cat Although precise figures cannot be given, if ampullary convergence were estimated from our present results, it would lie somewhere between 9 and 25% of second order canal neurons This again seems to be consistent with the results from the cat

Nerve endings of primary vestibular fibres in the cat (Gacek, 1969) and monkey (Stein & Carpenter, 1967) reveal certain topographical features Neurons innervating the ampullar receptor terminate in the rostral half and mid-portion of the vestibular nuclei complex and those innervating the macular receptor terminate in the caudal half and mid portion of the vestibular nucleus However, vestibular units responding to rotation did not show any such topographical distribution The units sensitive to ampullar stimulation were distributed diffusely throughout the vestibular nuclei complex Those sensitive to macular stimulation were less divergent and were distributed along the terminal parts of the primary fibres innervating the otoliths Such differing rates of divergence of ampullar and macular impulses in the vestibular nucleus seem to be reflected in other parts of the brain stem, such as the reticular formation (Speyer et al , 1974)

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ZUSAMMENFASSUNG

Unter Registrierung mit der Mikroelektrode von 181 Neuronen der Vestibulariskerne von Ratten wurden Horizontalbeschleunigungen und Kippungen in der Frontal- und Sagittalebene vorgenommen 68 dieser Einheiten sprachen auf Drehung und Kippung an (Kanal-Otolith-Zellen) Das heißt, 41% der Neuronen reagierten auf Drehung (d h 68/166) Der Auftrittsprozentsatz der Kanal Otolith-Zellen vom Neuronentyp I und II war unbedeutend, nämlich 48 6, beziehungsweise 37,0% Drehung um die senkrechte Achse mit geneigtem Kopf rief eine gleichzeitige Stimulation des Bogengangs und Maculae hervor Bei Anwendung einer solchen Reizmethode konnte der „bias"-Effekt bei 72,5% der Kanal-Otolith Zellen beobachtet werden (d h 29/40) Da die Drehung um die senkrechte Achse mit geneigtem Kopf auch eine Reaktion des vertikalen Bogenganges hervorrief, wurde außerdem der Grad der Ampullar-Konvergenz durch eine Analyse der Reaktion, die bei solchen Rotationen erreicht wurden, abgeschätzt Die Ergebnisse der Versuche an Ratten wurden mit den Ergebnissen von Versuchen an Versuchstieren anderer Arten verglichen

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RESULTS OF NEW AIR CALORIC TESTING METHOD AMONG NORMAL SUBJECTS

II *Monophasic Testing*

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Abstract A new caloric irrigation method is described In the new method the temperature of a continuous aural irrigation is switched between hot and cold values at precisely specified times The durations of hot and cold pulses have been calculated to produce specific caloric stimulation intensities based on known heat transmission characteristics of the labyrinth area A brief washout irrigation is used to eliminate promptly all caloric stimulation effects at the conclusion of each test The present study concentrated upon the question of how long the washout" pulse should be in order to obtain optimum results Repetitive application of a uniform stimulus intensity to 6 normal subjects indicated that shorter test intervals can be used without causing vestibular uation Prompt removal of the caloric stimulus can be plished by proper timing of the washout phase ıe new technique

At the present time clinicians rely primarily on caloric irrigations for assessing vestibular function (Wolfson 1973) and in recent years there have been efforts to improve the diagnostic usefulness of the caloric test by employing more accurate stimulation and response measurement techniques For the most part these efforts have focused on the careful control of irrigation fluid temperatures and recording of nystagmic eye movement responses for detailed analysis

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While such an approach has certainly helped to improve the effectiveness of clinical vestibular testing generally, there remain serious shortcomings For example, it is at present difficult to define consistent caloric response patterns whereby specific disease states can be recognized (Milojevic, 1966, Eviatar, 1972 Wolfson, 1973) Because responses to individual caloric stimulations exhibit a high variability, scores for individual tests are accorded only minor significance Interpretation of caloric vestibular test results has come to be confined mostly to a comparison of responses from the right vs left labyrinth and right beating vs left beating nystagmus, a procedure which is of limited value in following the course of disease or evaluating the effects of treatment

Response variability must be considered to be a serious obstacle to the straightforward assessment of vestibular function On the other hand, if stimulus intensity can be held constant response variations may be taken as a direct and easily observed manifestation of nervous system activity Thus the caloric test has a great potential utility for investigation and assessment of certain aspects of brain function (Collins et al 1961 Johner & Perlman 1968 Plum & Posner 1972) In any

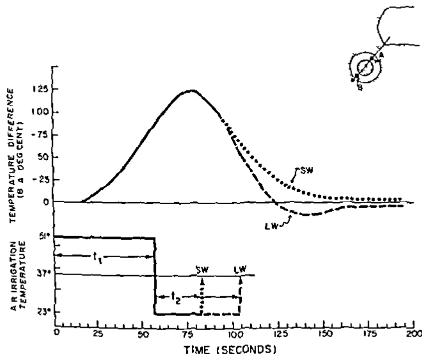


Fig 1 Upper graph shows time course of predicted temperature difference across the lateral semicircular canal (B–A) produced by a step temperature change during continuous aural irrigation with air. Lower graph shows time course of air irrigation temperatures required to produce "SHORT WASHOUT" (SW) and "LONG WASHOUT" (LW) effects (see text)

event, the development of practical and reliable methods for testing the peripheral or central vestibular systems will require an improved technique for controlling the intensity of the caloric stimulus

This report describes further evaluation of a new caloric irrigation method designed to maximize control over caloric stimulus intensity and to allow convenient and precise adjustment of stimulus intensity through a range of predetermined values In the new method the temperature of a continuous aural irrigation is switched between hot and cold values at precisely specified times The duration of hot and cold pulses have been calculated to produce specific caloric stimulation intensities, based on known heat transmission characteristics of the labyrinth area of the temporal bone A brief "washout" irrigation is used to eliminate promptly all caloric stimulation effects at the conclusion of each test

Theoretical calculations are discussed in greater detail elsewhere (Proctor et al , 1975, Proctor & Dix, 1975, Proctor et al 1976) For purposes of the present discussion it will suffice to point out that the nystagmic response to aural irrigations is generated almost entirely by the lateral semicircular canal receptor organ, and that the magnitude of the forces acting to move the cupula endolymph system of the lateral semicircular canal (i e , the "caloric stimulus") is nearly proportional to the temperature difference across this canal (Young, 1972) Therefore, in this report, we assume that the intensity of the caloric stimulus can be represented directly by the temperature difference across the lateral semicircular canal (B–A, Fig 1)

In previous studies we used water as the irrigation fluid, and demonstrated that nystagmic response intensity followed quite faithfully the roughly sinusoidal stimulus profile produced by a three-pulse temperature-switching irrigation sequence (Proctor et al , 1975, Proctor & Dix, 1975) A three pulse sequence using air as the irrigation 'fluid was shown to produce similar results, though certain differences between air and water had to be taken into account (Proctor et al , 1976) Our studies have also demonstrated that ca-

loric stimulus intensity can be adjusted reli ably when using the temperature-switching method, and that interaction among successive stimulations (order effect) did not occur when tests were applied at 10 and 6 minute intervals

The present study was carried out to explore two features of the new temperature switching air caloric test First, we needed more information concerning the manner in which the stimulus is terminated by the "wash out" irrigation, which is intended to reduce the temperature difference across the lateral semicircular canal to zero value as quickly as possible Second, we wanted to examine the test–retest stability of responses to a caloric stimulus whose intensity is presumably held constant among successive trials

METHOD

A two pulse irrigation sequence was selected that would produce only the hot phase of caloric stimulation, as shown in Fig 1 By irrigation with hot air for 58 seconds and then switching to a cold air WASHOUT pulse, the intensity of the caloric stimulus (B–A, Fig 1) should rise to a peak value of −1 25°C, and en promptly decline The duration of the SHOUT pulse was varied (SW vs LW, 1) to determine what effect this would ave on the terminal phase of the nystagmic response Irrigation sequences consisted of hot air for 58 sec followed by cold air for *either 25 sec (SHORT WASHOUT) or 45 sec* (LONG WASHOUT) It was anticipated that the SHORT WASHOUT (SW) irrigation sequence would be inadequate to terminate the nystagmic response promptly and that the LONG WASHOUT (LW) would have too great an effect resulting in a reversal of nystagmus in the terminal part of the reaction From the results of this study we hoped to extrapolate an optimal value for WASHOUT pulse duration

The two pulse air irrigation sequence was applied by means of two air–water heat exchangers During operation, this apparatus conducted dry air through either a warm or cold water jacket, depending on the setting of electromechanical air valves (Proctor et al 1976) Air temperature within the stimulator nozzle was displayed on a strip chart recorder along with the electronystagmographic record (ENG) of eye movements Irrigation temperatures were maintained during this experiment within 2 8% of set points Eye movement records were scored by identifying all nystagmus beats and measuring slow phase eye speed with a slope plotter as in previous work

Normal young adults were chosen who denied a history of ear disease, ear surgery, head injury, vertigo, motion sickness, or recent drinking, smoking, or medication Aural cross sectional dimensions were estimated by comparison with calibrated ear speculae and skull sizes were measured with calipers Body temperature of subjects and room temperature during the test sessions were recorded

Preliminary testing with ENG included ocular pursuit movements, mental alerting with eyes closed to test for spontaneous nystagmus, and positional testing, including the head hanging positions Subjects showing abnormal responses to these tests were excluded from the study During experimental irrigations, subjects were instructed to notify the tester of any discomfort or sensation of movement, and after each caloric stimulus they were asked to score their experience of vertigo on a 7-point scale (Lidval, 1961) Mental alertness was stimulated during the time of predicted peak responses for a period of 30 sec by having the subject perform mental arithmetic Variability of the corneoretinal potential was reduced to a minimum by having subjects keep their eyes open between irrigations and by performing the tests in a dimly lit room Eye movement calibration adjustment was performed immediately before and after each caloric irrigation

Following the preliminary evaluation four monophasic hot stimulations were applied to the right ear of each subject at 6 min inter

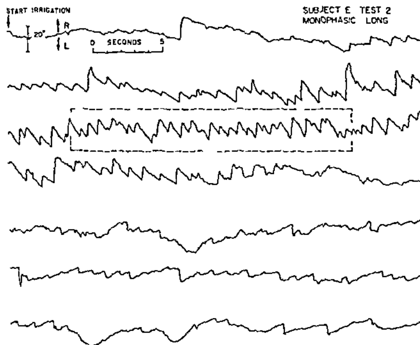


Fig 2 Response of normal subject to monophasic air caloric test, using LONG WASHOUT irrigation profile Each line of tracing represents 30 sec Broken lines indicate the 20 sec period when nystagmus was most intense Note reversal of nystagmus after about 125 sec

vals Three subjects were stimulated with SW irrigation sequences, and 3 received LW stimulations, assigning subjects randomly After a 20 min rest, four more monophasic stimulations were applied, reversing the assignment of SW and LW stimulations Following a 10 min rest, biphasic (hot/cold) stimulation at the same peak intensity (±1 25°C ΔT) was applied to the left ear, and 10 min later, to the right ear

RESULTS

Among these 6 normal subjects, the intensity and direction of induced nystagmus generally followed a time course close to the predicted stimulation curves of Fig 1 A typical example of the response to a LONG WASHOUT (LW) stimulus is shown in Fig 2, and a plot of mean slow phase eye speed during successive 5 sec samples of this response is shown in Fig 3

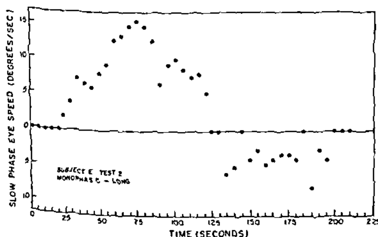


Fig 3 Plot of average slow phase eye speed during each 5 sec of response shown in Fig 2 Positive and negative eye speed values indicate nystagmus directed toward the same or opposite ear respectively

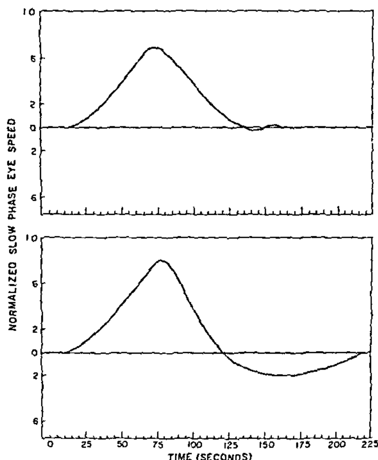


Fig 4 Average normalized eye speed scores for nystagmic responses of all subjects to SHORT WASHOUT (*above*) and LONG WASHOUT (*below*) stimulus profiles Shaded areas indicate one standard deviation of the data

As can be seen in these figures, nystagmus began to beat towards the irrigated ear after a lancy of about 20 sec Thereafter, the reaction ckly built up and reached a peak intensity about the same time as that predicted for he peak of the physical stimulus (82 sec), and then promptly diminished In this case, a nystagmus appeared after about 130 sec, directed away from the irrigated ear Such a reversed nystagmus during this time period is taken to indicate the LW irrigation sequence probably induced a brief reversal of the physical stimulus, as indicated tentatively in Fig 1 (LW, upper graph)

In order to compare the effect of LW vs SW stimulations upon nystagmus intensity curves, slow phase eye speed scores for all 48 monophasic tests (5 sec samples) were averaged for the two conditions In carrying out this computation, individual scores were divided by the highest score for that subject This procedure "normalized" each individual's scores with respect to his own maximum responsiveness thereby avoiding a disproportionate weighting of the response curve by any single individual The result of this analysis is shown in Fig 4 where solid lines indicate the course of mean slow phase eye speed and the shaded areas indicate one standard deviation It is clear that the LW irrigation sequence terminated the nystagmic reaction more promptly than the SW sequence However, the WASHOUT phase of the LW irrigations appeared to be too long, since it almost always caused a reversal of nystagmus Although this data provides a useful guideline, further work is needed before timing of the WASHOUT irrigation is definitively established (see Discussion, below)

Our evaluation of test–retest stability focused on measures that reflected either the overall or the peak intensity of individual responses

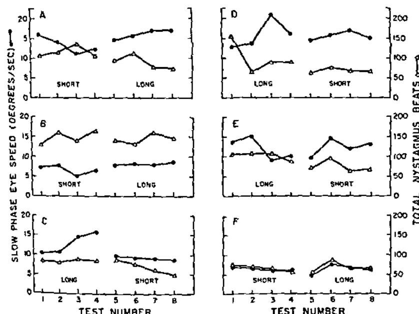


Fig 5 Slow phase eye speed averaged during the most intense 20 sec of each response (S E S 20), and total number of nystagmus beats during the entire response to monophasic caloric stimulations (see Fig 1) Four SHORT and four LONG WASHOUT irrigation profiles were used with each subject

S E S (5)
Maximum score for mean slow phase eye speed based on 5 sec sample periods

S E S (20)
Slow phase eye speed averaged during the most intense 20 sec segment of the response (outlined by broken lines, Fig 2)

Beats (20)
Total number of nystagmus beats during the most intense 20 sec segment of the response

Total beats
Total number of nystagmus in the appropriate direction

Of the above measures, the S E S (20) score appeared to be the most realistic and proper representation of response intensity Considering all 48 monophasic responses, there was a very close correlation between S E S (20) and S E S (5) scores, but much less agreement between S E S (20) and either BEATS (20) or TOTAL BEATS The poor correlation between measures of eye speed (S E S) and measures of frequency (beats) is for the most part an indication of inter-individual differences, rather than intra subject variance This relationship is well shown in Fig 5, where these two measures are seen to vary concordantly much of the time, despite the remarkable differences in their relative values among different subjects

A simple method for evaluating the data is shown in Table I, where responses to each of the 8 individual tests is averaged over 6 subjects It can be seen that both BEATS (20) and S E S (20) scores remain relatively stable as the test is repeated

A detailed statistical analysis was employed to examine formally the 8 monophasic test results with respect to inter- and intra- subject variation of response intensity and to determine whether varying the WASHOUT' period (LW vs SW) might affect response intensities S E S (20) was the only response measurement used The analysis indicated that there are large differences in the mean S E S (20) scores from subject to subject, and also significant fluctuations in the size of within subject variance This variance is lower for the SHORT WASHOUTS than for the LONG WASHOUTS For 3 of the 6 subjects (A, B, and C) there was a slightly higher mean S E S (20) score for the LW than for t

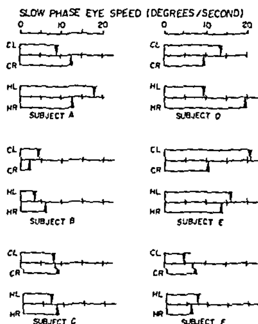


Fig 6 Responses to biphasic caloric stimulations (S E S 20) performed after completion of monophasic tests

SW responses A possible explanation for this is an upward trend in the responses when the LW stimulus is repeated, although this upward trend was not statistically significant for the data as a whole No evidence of an order effect was found for the SW trials (cf Fig 5 Table I)

has been reported (Henriksson et al , 1961) |the presence of unilaterally induced ves

habituation can be demonstrated not only by reduced reaction in the stimulated ear, but also by a directional preponderance of nystagmus opposite the habituated direction, when bithermal caloric tests are applied to either ear in the conventional manner To test for this effect, biphasic tests were applied to the left and then right ears, and responses r corded and scored as indicated above S E (20) scores were computed for the hot and co phase of each biphasic test and are show in Fig 6 The expected directional preponde ance of nystagmus (to the left in this cas was found only at a low level in subjects and F (−17 2% and −13 4%, respectively Moreover, subjects B, C, D, and E had dire tional preponderance to the right (28 65 13 1%, 26 7%, and 2 4%, respectively) Su ject E showed a right hypoexcitability (20 1% but subject D showed a left hypoexcitabili (−11 1%) Other hypoexcitability scores we at the 5% level or lower Thus consistent e dence of vestibular habituation or unilater hypoexcitability following the series of mon phasic stimulations is not shown by this dat Furthermore, there was only a slight decrea in average S E S (20) scores for the enti group, when the 8 monophasic tests ($\bar{x}$, 11 21 sec) are compared with the hot phase of th succeeding biphasic tests ($\bar{x}$, 10 56°/sec)

Subjective experiences during monophas responses are shown in Table II There was 20 min resting period between tests 4 and but otherwise tests were applied at 6 min inte vals Only one subject (E) experienced an u pleasant sensation of vertigo, and none e perienced nausea Estimations of vertigo in tensity, based on a seven point scale (Lidva 1961), varied considerably both within ar among subjects The first and fifth irrigation produced significantly higher vertigo score and there was a positive correlation betwee vertigo scores and S E S (20) scores

Ear canals in this group varied between 4 2

Table I *Nystagmus intensity scores grouped according to caloric stimulation profile (SHOR vs LONG WASHOUT)*

Each value is the average of 6 tests (one test score from each subject)

Subject	Short washout tests				Long washout tests			
A B F	1	2	3	4	5	6	7	8
C D E	5	6	7	8	1	2	3	4
Beats 20	27 17	26 33	23 17	24 33	24 50	26 17	30 17	24 50
S E S 20	10 68	11 31	10 14	10 28	10 69	11 73	12 61	12 25

Table II *Subjective experiences during monophasic air caloric tests (8 tests applied to each of 6 subjects)*

	Subjects					
	A	B	C	D	E	F
Sense of Movement	Yes	Yes	Yes	Yes	Yes	Yes
Spinning	Yes	No	Yes	No	Yes	Yes
Unpleasant (0 to +++)	0	0	0	0	+	0
Nausea	0	0	0	0	0	0
$\bar{X}$ Vertigo score (0–7)	1 87	2 87	2 75	1 37	4 12	3 12

and 6 5 mm (smallest cross sectional diameter) and there was a positive correlation (0 97) between S E S (20) and ear canal size However, considering the small sample size and the lack of correlation of these measures in our previous studies, no final conclusions can be drawn at this time There was no significant correlation between S E S (20) and anterior-posterior skull size (range = 18 0–20 2 cm) inter mastoid skull size (13 4–14 8 cm), age (19–24 years), subject temperature (36 3°C–37 4°C) room temperature (26 3°C–27 0°C) or sex (2 females, 4 males)

DISCUSSION

Both the detailed statistical analysis and bithermal testing demonstrated that the new temperature switching procedure allowed repetition of caloric stimulations at reduced inter test intervals without vestibular habituation This contrasts with the results of Henriksson et al (1961), and Lidval (1961) who showed strong habituation effects with inter-test intervals of 5 and 10 min, respectively, using conventional caloric stimulation methods Our data showed more variability and a slight progressive increase in intensity of responses when LW stimulations were used, compared with SW stimulations This finding indicates that the exact manner in which the caloric stimulus is terminated also may be an important factor in controlling the effects of repetitive stimulations

The present study indicates that the SW irrigation sequence would be superior to the LW sequence for clinical purposes, because of the presumably undesirable period of reversed nystagmus (Fig 4) and the slightly greater variability to be expected with use of the LW sequence For the present, we feel the SW sequence is an acceptable approximation of the proper temporal relationship between the primary hot irrigation (t_1) and the succeeding WASHOUT *irrigation* (t_2)

However, it must be pointed out that certain discrepancies are to be expected when inferring caloric stimulus magnitude from nystagmic response intensities It has been amply demonstrated that the relationship between nystagmus intensity and the intensity of the physical stimulus acting on the semicircular canal receptor is a highly modifiable one (Lidval, 1961, Henriksson et al , 1961, Collins et al 1961, Johner & Perlman, 1968, Plum & Posner, 1972, Benson, 1974) Following conventional caloric stimulation, the diminution and final termination of nystagmus is thought to be heavily influenced by neural adaptive processes (Benson, 1974) Thus, the reversed nystagmus found with LW irrigations in this study may very well have resulted from the action of such adaptive processes rather than an actual reversal of physical forces acting on the cupula By the same token it is not possible to determine what portion of intra subject response variability was caused by variations in stimulus intensity and what portion was due to imposed alterations in responsiveness of the vestibulo-ocular reflex The resolution of this subtle and somewhat paradoxical difficulty may be best approached through further intra

labyrinthine temperature studies (Young, 1972)

Further work is needed to demonstrate what variations may be expected in the intensity and form of the nystagmus response, when the new temperature-switching procedure is applied to subjects with grossly deviant aural canal and skull sizes, or with significant alterations in temporal bone dimensions Our preliminary calculations and experiences to date do not lead us to expect great distortions of the stimulus profile on this account Furthermore, so long as a particular individual s physical dimensions remain constant, there is no reason to expect significant variation in the intensity of the caloric stimulations produced by successive applications of the new irrigation technique to the same subject

The new temperature switching air caloric irrigation technique offers several advantages over conventional methods A previous report has demonstrated how the intensity of caloric stimulation can be adjusted in a convenient and reliable manner (Proctor et al , 1976) The present report provides evidence that a given intensity can be reproduced within the same subject even when relatively short inter test intervals are used (Fig 5) Finally, by using a ASHOUT irrigation to remove the caloric timulation quickly, the practical advantages are achieved of reducing the duration of the test subject s discomfort and reducing the waiting time between tests

ZUSAMMENFASSUNG

Eine neue Technik fur die kalorische Reizung des Vestibularisrezeptors ist hier beschrieben Der Prozeß ist so entworfen daß eine maximale Kontrolle uber die Starke der kalorischen Reizung erzielt werden kann Er erlaubt eine bequeme und genaue Festsetzung der Reizungsintensität in dem Raum von vorherbestimmten Werten In der neuen Methode ist eine ununterbrochene Ohrenspulung an einer genau spezifierten Zeit von heißen zu kalten Temperaturen geschaltet Die Dauer des heißen Pulses wurde berechnet um genaue kalorische Stimulationsintensitaten zu erzielen die auf schon bekannte Warmeleitungseigenschaften des Schlafenbeines basiert sind Wie schon fruher beschrieben wurde der heiße Puls mit einer kurzen kalten Ausspulung beendet und dieser kurze Puls erzeugt gleichzeitig eine Reizauswaschung In der vorliegenden Arbeit konzentrien sich auf die Frage wie lange der Reizauswaschi Puls wahren soll um die besten Resultate her bringen Die wiederholte Anwendung einer gleichm Stimulationintensitat an sechs normalen Versuchs nen zeigte daß man kurze Zwischenversuchs haben kann ohne eine vestibulare Gewöhnung zu sachen Eine sofortige Entfernung der kalorische zung wird erreicht durch eine sinngemäße Regelu Reizauswaschungs Phase der neuen Techn k

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HORSERADISH PEROXIDASE ACUTE OTOTOXICITY AND THE UPTAKE AND MOVEMENT OF THE PEROXIDASE IN THE AUDITORY SYSTEM OF THE GUINEA PIG

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Abstract When guinea pig cochlea was perfused *in vivo* with a solution of 1% horseradish peroxidase (HRP) in artificial perilymph the enzyme was found in the basilar membrane spiral limbus some outer and inner hair cells and some supporting cells and it was gradually cleared away with time Acute signs of cell damage included *swelling* vacuolization and diffuse labeling of some hair cells but stereocilia remained normal in configuration Albino melanocytes of the spiral ligament were also damaged and vacuolization of Reissner s membrane occurred after 10% HRP Both concentrations caused a gradual decline in CM showing that HRP is acutely ototoxic but its mode of action is unknown No retrograde transport of HRP to spiral ganglion cells or to brain stem neurons occurred but some brain stem neurons took up HRP from the neuropil following diffusion from the cochlea

Our study of horseradish peroxidase (HRP) uptake and movement in the auditory system of the guinea pig was undertaken in response to the published reports of Gacek & Lyon (1974) and of Warr (1975) who claim to have located brain stem sources for the vestibular and cochlear efferents in the kitten by the HRP retrograde transport method Their work is subject to a number of criticisms briefly considered below which cast some doubt on the validity of their results The scope of our own work with HRP broadened considerably, however, as we happened upon numerous ancillary findings in the course of our investigation which we believe are worthwhile including in the present report

In tracing the evolution of our study it is necessary first to examine the grounds for considering the prior work of Gacek & Lyon (1974) and of Warr (1975) questionable One of the foremost objections centers around their nearly exclusive use of newborn kittens as the basis of their accounts It is well known that the inner ear of the newborn kitten (and of such other commonly used experimental animals as the rat mouse and dog) is neither morphologically nor physiologically mature the blood–brain barrier is not adult in type and other possible barriers to diffusion of large molecules toward the nerve terminals and fibers are present which do not exist in the adult For example, the fluid filled spaces of the organ of Corti which might facilitate movement of HRP to the nerve fibers, are not present at birth Neither is the efferent innervation itself established (Kikuchi & Hilding 1965) The organ of Corti of the cat does not reach maturity until some time between days twelve and fifteen (Pujol & Marty 1970)

Moreover, the incompletely developed neurons and nerve terminals of such essentially fetal inner ears may be incapable of retrograde

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transport of HRP Jacobson & Trojanowski (1975) have shown that the cortical neurons of the rat, which undergo postnatal maturation similar to that of cortical neurons of the kitten (Purpura et al , 1963), cannot be labeled by the retrograde transport of HRP earlier than 4 or 5 days postnatally The typical adult pattern of HRP uptake first occurs between the eighth and fifteenth day Although Jacobson & Trojanowski s findings in cortical neurons of the rat cannot be extrapolated *en masse* to other parts of the nervous system which undergo similar periods of postnatal development, nor to other species, still they suggest that transport phenomena may not be demonstrable in immature neuronal populations until a certain level of maturation has been achieved

Warr's findings, which most concern us here because our own efforts have been directed toward the auditory and not the vestibular system, must be assessed for validity for other reasons only 5 out of 48 experimental animals were selected for his report, the labeling of neurons unrelated to the auditory or vestibular systems precludes experimental specificity, and no proof of peripheral uptake was offered The last named objection is paramount, because Duvall & Sutherland (1972) Jahnke 72) and de Lorenzo et al (1973), who stud HRP distribution in the cochlear labyrinth fter short and long survival times, did not find the protein inside nerve terminals or nerve *fibers within the organ of Corti* Neither was the enzyme present in nerve fibers at the habenula perforata or proximal to it

The present research was begun then in order to restudy cochlear uptake of HRP and to determine whether or not Warr s results could be duplicated in young animals which had a mature auditory system and blood-brain barrier from birth Artificial perilymph rather than saline was employed as the vehicle, and a closed perfusion system was used which did not allow back spillage into the middle ear It was to such back spillage that Warr attributed labeling of neurons outside the "efferent" system It was said that fibers of passage in the middle ear had taken up the spilled HRP, although earlier, purposeful injection of the middle ear by Gacek & Lyon (1974) had failed to demonstrate such uptake and retrograde transport

We believed our method to be more physiological In order to test for this, however, we studied cochlear microphonic (CM) activity before, during and following cochlear perfusions with HRP or with the vehicle alone To our surprise, the CM recordings suggested that HRP was acutely ototoxic, a finding we then explored further as the scope of our research widened

This report deals, then, with our electrical and morphological findings concerning HRP ototoxicity, HRP uptake into cochlear structures, and the occasional diffusion of HRP along the eighth nerve to the brain stem where it is then taken up from the neuropil into neurons comparable to those reported by Warr (1975) An incidental finding the labeling of albino melanocytes (for terminology see Della Porta & Muhlbock, 1966, Niebauer, 1968) by HRP, which reveals a distribution similar to that found in pigmented animals, will also be considered

MATERIAL AND METHODS

Fourteen healthy guinea pigs ranging between 250 and 385 g were anesthetized with Dial (0 8 *ml/kg body weight) and placed on a respirator* for artificial ventilation through a tracheotomy tube The bulla was surgically exposed ventrally, opened, and two small holes were made in the cochlea at the basal turn one in scala tympani and the other in scala vestibuli Cochlear perilymphatic perfusion with artificial perilymph was established according to the procedure of Nuttall et al (1976) Gravity flow of the artificial perilymph was adjusted to the rate of about one drop/min (~15 μl/drop) Heart rate was monitored electrocardiographically throughout the experiment and (rectal) temperature was kept at about 36°C with the aid of a heating pad

HRP experiments

In twelve experiments a 1% (10 mg/ml) solution of HRP (Sigma Type VI) in artificial perilymph was then substituted as the perfusate It was allowed to flow for one hour in 5 cases, whereupon the experiment was terminated In 6 of the remaining 7 cases, HRP solution was perfused for one hour and the animals were permitted to survive for 7 (2 cases), 11 (2 cases), and 23 (2 cases) additional hours before sacrifice One animal from the last named group received six more drops of the peroxidase immediately prior to cochlear fixation In the one remaining experiment, only ~63 μl of 1% HRP perfusate were introduced, this animal was allowed to survive for a total of 24 hours from the beginning of perfusion

One experiment was carried out utilizing a 10% solution of HRP The animal used had already been perfused with ~93 μl of artificial perilymph in a control experiment, during and after which time CM was recorded After the CM had restabilized, this guinea pig was perfused with 0 5 ml of 10% HRP in artificial perilymph over a period of about one hour and was permitted to survive for 1½ additional hours, while CM was recorded

Controls

To discover possible deleterious effects of the perfusion itself, artificial perilymph was substituted as the perfusate, mimicking substitution of HRP as outlined above In one case, the artificial perilymph was perfused for one hour and the animal was allowed to survive for 3 additional hours The CM was recorded before, during and following perfusion In another case, only ~93 μl of artificial perilymph were allowed to perfuse before stop-flow occurred CM was recorded before, during, and for about one hour following perfusion This same animal was used in the 10% HRP experiment described above

Electrophysiological measurements

Cochlear ac potential (cochlear microphonic, CM), in response to a continuous 4 kHz acoustic stimulus, was recorded from seven of the perfused ears Experiments were conducted in an electrically-shielded sound-isolation booth

Sound was delivered to the external auditory meatus by way of a closed sound system, and the sound intensity was adjusted to result in an initial CM magnitude (during the start of perilymphatic perfusion) of 8 μV Previous sound calibration established that this electrical output, from a normal animal, results from a sound intensity of approximately 70 dB SPL (sound-pressure level)

CM signals were taken from the micropipette in scala tympani which thus had a dual role, serving both for perfusion and for recording The single-ended signal (reference to a neck muscle ground) was preamplified (Princeton Applied Research Co Amplifier Model CR-4A) and measured on a Hewlett Packard Co Model 302A wave analyzer Signal magnitude was then continuously recorded on a Grass Instrument Co Model 7 chart recorder

Fixation

In all experiments involving post perfusion survival, the cochlea on the experimental side was fixed by perilymphatic perfusion of a freshly-prepared, cacodylate buffered (pH 7 2) 1% glutaraldehyde, 4% paraformaldehyde solution, while the animal was still on the respirator The guinea pig was then perfused intravascularly by the intracardiac route with physiological saline, followed by the fixative described above The second cochlea was fixed perilymphatically after the whole body perfusion, as were both cochleas in the experiments which were terminated immediately after perfusion

The fixative was chosen because of its excellent qualities for preservation of neural structures for ultrastructural as well as light microscopical studies Some recent investigations (Jones & Leavitt 1974 Kim & Strick [illegible]

ever obtained satisfactory demonstration of the protein after fixation in mixtures containing 2% paraformalde

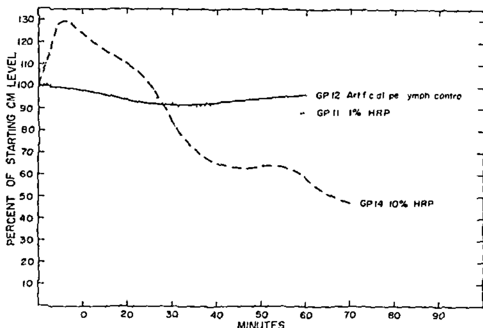


Fig 1 The changes in the magnitude of the CM (at 4 kHz) which occurred *during* periods of continuous perilymphatic perfusion with artificial perilymph and with two concentrations of HRP in artificial perilymph are shown in the above graph The relative increase (to 130%) in the earl part of the 10% HRP curve is an artifact of the method due to viscosity (see text)

hyde These data are in conflict with each other and with results obtained earlier by many investigators including Kristensson & Olsson (1971) who first described retrograde transport of HRP and used Karnovsky s fixative (1965) which contains 4% paraformaldehyde The issue of paraformaldehyde concentration for optimal preservation of HRP in neurons to which it has been transported annot be considered to be settled In this series of experiments we have chosen to keep the parameter of ative concentration constant

Following perilymphatic and intravascular perfusion of the fixative, all extraneous tissue was removed from the head and the skull was carefully opened The exposed brain was washed with additional fixative several times as dissection proceeded The cerebral hemispheres were removed the brain rostral to the midbrain was cut away and the brain stem was widely exposed to permit more thorough contact with the fixative The brain and inner ears *in situ* were then left in additional fixative overnight at 4°C The following morning the brain was removed from the skull trimmed, and sectioned at 25 μm on a freezing microtome The inner ears were microdissected pieces of the organ of Corti spiral ligament and stria vascularis, spiral ganglion and acoustic nerve were collected in buffer The brain stem sections and pieces of the inner ears were then treated histochemically for demonstration of HRP

Histochemical procedure

The procedure followed for demonstration of sites of HRP uptake was that of Graham & Karnovsky (1966) The histochemically prepared tissues were mostly mounted in glycerol for bright field and phase contrast light microscopic study, but some were post fixed stained with osmium and embedded for future electron microscopical investigation A few brain stem sections were counterstained with cresyl violet and mounted in Permount The bulk of our histochemically prepared tissue however was studied and photographed directly without counterstaining

RESULTS

In the following descriptions and in the discussion the terms reaction product labeling and staining are used interchangeably

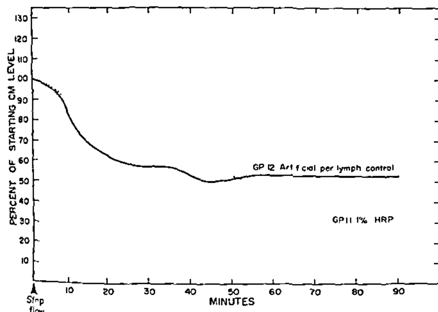


Fig 2 The above graph illustrates changes in the magnitude of the CM (at 4 kHz) which took place *after* periods of more than 60 min of continuous perilymphatic perfusion during which time there was no perfusate flow The curves have been renormalized to the CM level at the start of the stop-flow condition (arrowhead lower left)

Cochlear Microphonic Recordings

CM recordings during and following perfusion with 1 and 10% HRP in artificial perilymph and with artificial perilymph alone are presented in Figs 1–3 During continuous perfusion with the HRP (Fig 1), the magnitude of CM was gradually depressed relative to that in the control perfusion[1] (GP12 artificial perilymph alone) The higher concentration of HRP (10%) produced the greater reduction of CM although even the 1% HRP solution caused some microphonic loss (Fig 1) The early increase in CM to values greater than the starting level (10% HRP Fig 1) is an artifact of the method related to viscosity controlled pressure changes which cause a base line shift of the CM level

If the control perfusate flow is halted after periods of continuous perfusion CM will first undergo a reduction and then tend to stabilize at a new level This is illustrated by the control perfusions in Figs 2 (GP12) and 3 (GP14) We believe that this method related CM reduction is a function of the oxygenation of the artificial perfusate relative to the natural perilymph which is being replenished during the stop flow condition Nevertheless, when the perfusate contained HRP, the reduction in CM was greater during the stop flow recording period (Fig 2, GP11) The least damaging to the CM (and thus the most physiological condition) was the low concentration of HRP (1%) administered in ~63 μl, approximately 6 min of perfusion time, as shown by the function for GP13 in Fig 3 Note that all stop-flow curves in Figs 2 and 3 have been renormalized to the initial stop-flow magnitude for ease of comparison

Cochlear Structures

Short term survival animals

All perfusions with 1% HRP for one hour with termination of the experiment immediately

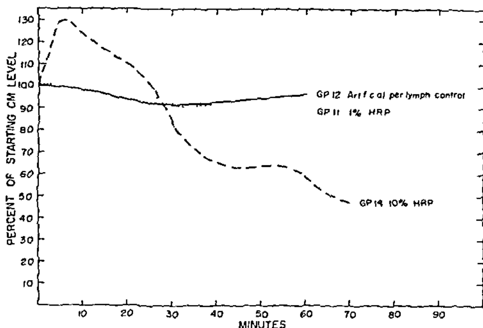


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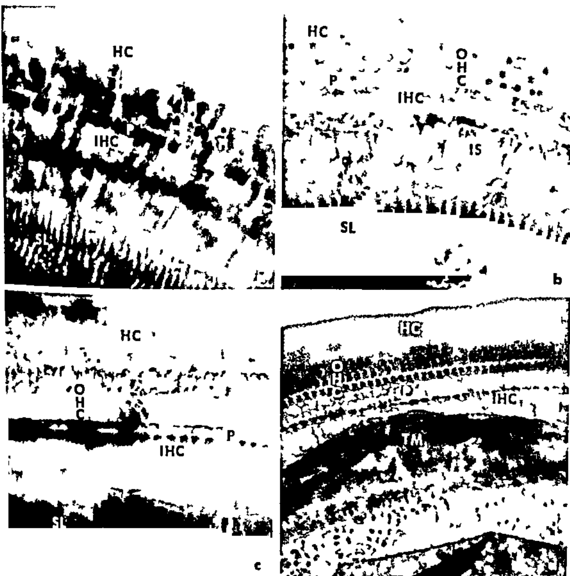


Fig 4 The deposit on of HRP n the organ of Cort and rela ed structures wh ch occurred under various experi mental cond ons s compared here A sound st mulus was not appl ed to the cochlea n the case of Fig 4a but was p esented n the rema n ng cases illustrated Fig 4a demon trates the variable uptake observed mmed ately follow ng 1 hou perfus on w h 1% HRP (second cochlear turn) Gradual clearing occurred after 7 hours (Fig 4b th rd cochlear turn) Twenty four hours after perfus on w th ~63 μl 1% HRP rema n ng depos ts of HRP were largely granular (Fig 4c second cochlear turn) Fig 4d (second cochlear turn) illustrate the ntense label ng observed after 1 hour perfus on with 10% HRP 1½ hours add t onal surv val time *HC* Hensen cells *IHC* nner hair cells *IS* nner sulcus *OHC* outer ha r cells *P* p llar cells *SL* sp ral l mbus *TM* tectorial membrane Fig 4a ×3 0 Fig 4b d × 00

The outer hair cells were more variable After only 7 hours certain outer hair cells were still more heavily labeled than others (Fig 4b) After greater periods of time however the d ffuse stain ng was replaced by granular de pos ts of react on product The hairs of neither the outer nor the inner hair cells were defined by reaction product Under immersion oil and with phase m croscopy however it was de termined that the hairs were present and gen erally of typical configuration

In the case of the animal whose cochlea had

been perfused with six more drops of HRP immediately prior to fixation, the distribution of reaction product was much the same as in our short-survival animals That is, the inner ear structures in general appeared to respond as though this were an initial contact with the protein Certain supporting and hair cells were more heavily labeled than others, and the spiral limbus, basilar membrane, spiral ligament and stria were diffusely and intensely stained

~63 µl of HRP

Perfusion of ~63 µl of 1% HRP succeeded by approximately 24 hours survival time resulted in diffuse, but diminished, staining of the spiral limbus, stria vascularis, and spiral ligament Infiltration of the tectorial membrane was slight The deposits of reaction product in Hensen and inner sulcus cells were granular rather than diffuse (Fig 4c) Both the inner and the outer hair cells contained granules of reaction product, with the greater deposits in the inner hair cells (Fig 4c) The hairs of the receptor cells lacked reaction product around or in them and could be visualized only under oil and with phase microscopy, they appeared to be typical in configuration

% HRP perfusion

rfusion with 10% HRP for one hour resulted the most intense staining of cochlear structures observed in our experimental series (Fig 4d) Except for the amount of reaction product present which was increased the results were generally similar to those obtained in short term experiments utilizing lesser concentrations of the peroxidase The reaction product was diffuse and intense in the stria vascularis spiral ligament spiral limbus and basilar membrane The tectorial membrane was heavily infiltrated Most of the outer hair cells were darkly labeled (Fig 4d) the inner hair cells showed both granular deposits and diffuse labeling The hairs of the receptor elements were well defined by deposits of reaction product in this case One noteworthy difference observed in this animal from our other experimental specimens was the presence of heavy labeling in the nuclei of cells of the mesothelial layer of the vestibular membrane (the cytoplasm was not evident) and differential staining of the cells of the epithelial layer Patches of epithelial cells were diffusely filled with reaction product, such cells also often appeared vacuolar

Albino Melanocytes

The albino melanocytes were not visible in non-perfused inner ears, nor in controls stained with osmium Neither could the melanocytes be distinguished in short survival-time animals nor in those perfused with 10% HRP This was most likely due to the intense background labeling which occurred in most places in which the cells are commonly found As clearing of the cochlea took place, however, the melanocytes became evident, particularly on the scala vestibuli and scala tympani sides of the stria vascularis, along the blood vessels there Other melanocytes were present near the blood vessels proximal to the vas spirale, in the osseous spiral lamina along the crest of the spiral ligament where the basilar membrane attaches lateralward and scattered along the eighth nerve in the modiolar canal

The albino melanocytes were many branched and were darkly stained by the histochemical procedure The cytoplasm of many of the cells looked vacuolar, or foamy (Fig 5a and inset), and the ends of the processes were often bulbous 24 hours after perfusion with 1% HRP In other cases, the processes of the cells seemed to have been disrupted and the albino melanocytes appeared to be in stages of disintegration

In the case of the animal perfused with ~63 µl of HRP the albino melanocytes appeared to be more numerous near the stria and less vacuolated The very fine processes of these cells could now be seen to enwrap blood vessels (Fig 5b) and sometimes contained small granules of reaction product Aside from

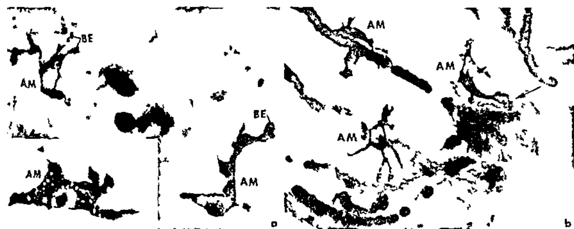


Fig 5 Figure 5a and its inset illustrate the vacuolization of the albino melanocytes (*AM*) and bulbous enlargement (*BE*) of their cell processes observed after 1 hour's perfusion with 1% HRP 7 more hours survival time As shown in Fig 5b less cell damage was evident in another animal perfused with ~63 μl 1% HRP and allowed to survive for 24 hours Many cell processes were fine (*FP*) and often wrapped around blood vessels (arrow center right) Fig 5a ×500 inset ×800 both figures illustrate cells at the vestibular border of the spiral ligament Fig 5b ×500 cells from the tympanic border of the spiral ligament

melanocytes some pericytes along the blood vessels in the spiral ganglion and elsewhere took up HRP

Eighth Nerve Fibers

The nerve fibers of the spiral bundles and those crossing the tunnel in the organ of Corti were often visualized in short survival animals due to their coating of HRP as were the fibers at the foramina nervosa and in the osseous spiral lamina In cross section in the few instances in which the angle of section was appropriate the fibers did not appear to contain the protein The nerve fibers within the spiral ganglion and modiolus also were coated with reaction product but the product was never detected around the nerve fibers proximal to the internal auditory meatus unless retrograde diffusion (see below) had also occurred This suggested the existence of a barrier to diffusion of HRP toward the brain stem but we could find no clear anatomical line of demarcation between nerve segments coated with reaction product and those lacking such deposits in our present material

Spiral Ganglion

The myelin sheaths and Schwann cell coverings of the spiral ganglion cells always appeared to be stained in freshly prepared histochemically treated segments of spiral ganglion None of the cells took up HRP into their cytoplasm as clearly seen in cross sections except for the unmyelinated neurons in our preparation perfused with ~63 μl of HRP (Fig 6) In this case all of the unmyelinated neurons in the available piece of ganglion were labeled

Brain stem neurons

No retrograde transport of HRP from the cochlea to the perikarya in the central nervous system occurred in our present series of experiments In three of our early experimental animals with short survival times diffusion of the peroxidase to the brain stem took place as ascertained by the presence of reaction product around eighth nerve fibers up to the medulla oblongata and by the staining of the margins of the brain stem The reason for this diffusion is obscure

In only one instance was the diffusion marked in amount (Fig 7) and in this case the protein was taken up into neurons apparently from the extracellular spaces of the neuropil The labeled neurons differed from one place to another both with respect to degree of staining and to the physical appearance



Fig 6 Unmyelinated neu rons (*UN*) were labeled after exposure to ~63 μl 1% HRP Only the sheaths of the mye linated neurons (*MN*) took up the enzyme Total sur vival time 24 hours ×800

of the reaction product they contained In those cells which were only lightly stained, the reaction product often appeared to be diffusely distributed in the cytoplasm In moderately or heavily labeled neurons, the reaction product was often diffuse but, at times, granular The granules were generally large in the case of the most intensely stained neurons (Fig 8) The nucleus was clearly visible within the labeled eurons almost without exception

ells containing reaction product were d on both sides of the brain stem, particu rly near its base The most numerous of these were found on the side ipsilateral to the perfusion, where diffusion into the brain stem parenchyma was also most extensive The specific cell groups showing most intense HRP uptake were very similar on the two sides, however These included the more ventral subnuclear groups of the facial motor nucleus, reticular neurons near those subgroups and others scattered more dorsally in the teg mentum, periolivary cells, particularly those dorsal and dorsolateral to the hilus of the su perior olive, some neurons of the superior olive and a few cells of the accessory superior olive and some neurons of the medial trape zoid nucleus A few neurons lying at the ventromedial border of the lateral vestibular nucleus and a very few more lying closer to the floor of the ventricle were lightly labeled on the ipsilateral side Some of the neurons of the ventral and many cells of the dorsal cochlear nuclei were intensely labeled, but only on the side ipsilateral to the perfusion

It is worth noting that numerous neurons di rectly in the path of the diffusion showed little or no reaction product within them The neu rons of the trapezoid gray lying ventral to the superior olivary complex were in a region heavily infiltrated with peroxidase Their peri karya often were studded with small, ring like arrangements of reaction product, as though the peroxidase had filled interstices between glial or neural endings located there Never theless, the perikarya themselves were re markably free of reaction product On the other hand, some of the labeled reticular neu rons lay outside the area of visible diffusion

Aside from neurons, some pericytes also took up the peroxidase The walls of the blood vessels lying near the base of the brain stem often were diffusely stained

Controls

Cochlear structures on the side contralateral to the perfusion with HRP did not show reac tion product within them The red blood cells



ig 7 Diffusion of HRP to the brain stem resulted in ptake into some neurons of the superior olive (*SO*) ccessory superior olive (*ASO* note arrow) and medial apezoid nucleus (*MTN*) Periolivary cells (*PO*) dorsal nd dorsolateral to the dorsal hilus (*DH*) of the SO were so labeled Neurons of the trapezoid gray which lay entral to the superior olivary complex although in the path of diffusion of HRP were not labeled (two arrows lower center) The section was rotated for photographic purposes the midline is indicated by the long arrow at the left *Nuc Sp Tr V* nucleus of the spinal tract of V *TB* trapezoid body One hour s perfusion with 1% HRP immediate sacrifice Section at the level of the cochlear nerve lightly counterstained with cresyl violet ×50

hen present, were labeled, however, due to he presence of endogenous peroxidase

DISCUSSION

Our light microscopical findings concerning he distribution of HRP reaction product in cochlear structures are in agreement with the esults of Jahnke (1972), Duvall & Sutherland 1972) and de Lorenzo et al (1973), who took heir studies to an ultrastructural level Our esults further indicate that the outer and inner hair cells, although *demonstrating some swelling or vacuolization* during short term exposure to HRP, are not damaged to the point of disintegration after 24 hours (see also Duvall & Sutherland, 1972) The stereocilia are generally of normal configuration The short term adverse effects of HRP are considered further below, but the anatomical findings in the organ of Corti 24 hours after exposure to a low concentration of HRP are not, in themselves suggestive of chronic ototoxicity

There are two additional anatomical indicators of acute HRP ototoxicity to be considered, however These are damage to the albino melanocytes of the spiral ligament in our 24 hour experiments utilizing 1% HRP, and vacuolization of Reissner s membrane in our *short term 10% HRP experiment* Our morphological evidence of acute HRP ototoxicity has been substantiated by our CM results which demonstrate that HRP depresses normal electrical activity in the cochlea



Fig 8 HRP reaction product was in the form of granules in heavily labeled brain stem neurons Periolivary cells section lightly counterstained with cresyl violet ×800

It is possible that at least part of the adverse effect of HRP might be colloidal osmotic On the other hand, HRP is a basic protein and basic proteins are known to be toxic to epithelial cells (Quinton & Philpott, 1973, Seiler et al , 1975) When compared with the known lectrical and anatomical effects of the oto- ic aminoglycosides neomycin and kana- /cin, HRP seems to resemble these drugs in s acute action The CM is depressed in a dose dependent way by both neomycin (Nuttall et al , 1976) and HRP, and vacuolization of Reissner s membrane has been reported in guinea pigs and monkeys following exposure to neomycin or kanamycin (Hawkins, 1970, Kaneko et al , 1970) and streptomycin (Nowak, 1974) Attention has also been called to the importance of the spiral ligament and strial cells for the integrity of the hair cells (Hawkins, 1970) and we have found the albino melanocytes of the spiral ligament vulnerable to damage by HRP However, in a single experiment, bovine serum albumin also depressed the CM, and far more rapidly than did HRP of similar concentration (Ross & Nuttall unpublished findings) Bovine serum albumin is not a basic protein but contains free sulfhydryl groups It is clear that much more investigative work is essential to establish the mode and site of action of both HRP and the albumin on cochlear function

The short term effects of HRP, including the swelling of some hair cells and the generally diffuse (rather than granular) appearance of the reaction product within many cells of the organ of Corti initially, are signs of some degree of cell injury Why some cells were more infiltrated with HRP than other, neighboring cells in our experimental series is at present unknown Note that these effects were observed in our non acoustic-stimulated ears as well as in those that received the low level of sound stimulus for CM generation

The direct effects of HRP on the hair cells and supporting cells of the organ of Corti appear to be reversible according to our results because HRP is gradually cleared from them with time and is reduced to granular deposits after 24 hrs The albino melanocytes, however, sometimes suffer permanent damage and disintegrate after exposure to the basic protein Our findings further indicate that HRP has a deleterious effect upon the melanocytes of the spiral ligament when applied in volume and concentrations that do not cause vacuolization of Reissner's membrane The albino melanocytes appear to be the most vulnerable of all cochlear structures to the basic protein HRP

Although we do not have direct experimental evidence concerning the chronic effect of HRP perfusion on either the cochlear morphology or the CM (i e , either recovery or continued depression of the microphonic), the available data lead us to speculate that full functional recovery of the organ of Corti could be compromised by the long term effects of injury to Reissner's membrane or to the albino melanocytes The effects on the CM would likely be complex but one would expect that the CM depression would in the long run be permanent

Turning now to our retrograde transport

studies, we have been unable to demonstrate such transport to brain stem neurons in the weanling or young guinea pig We have found that under some conditions diffusion toward the brain stem can occur, causing a seepage of HRP into the extracellular spaces of the neuropil When the seepage is great enough, certain cells within and near the area of diffusion and others farther away, can take the enzyme up, while others apparently do not It is of interest that the neurons we have found to have a proclivity for HRP uptake from extracellular spaces correspond greatly with those described by Warr as labeled by retrograde transport of HRP under his experimental conditions It is of further interest that the uptake we observed was greater on the ipsilateral side Warr, in contrast to all who have worked on the so called efferent olivocochlear and vestibular systems, placed the major source of the efferents on the side ipsilateral to the perfusion

That such direct uptake of exogenous HRP by intact neuronal perikarya and/or their dendrites is possible has been adequately demonstrated by others (Becker et al , 1968, Holtzman & Peterson, 1969) The intracellular distribution of reaction product was similar to that seen after uptake from axonal terminals

Why diffusion back to the brain stem did not occur in every experiment is enigmatic HRP has ready access to the fluid filled spaces around individual eighth nerve fibers and spiral ganglion cells Here the protein is in locations corresponding to endoneural and sub arachnoid spaces and no further cellular barrier is interposed between the peroxidase and the brain stem parenchyma (Shanthan & Bourne, 1968, Nabeshima et al , 1975) Theoretically, the peroxidase could have diffused toward the brain stem but it did not except under the unusual circumstances dealt with above A barrier to such diffusion must normally exist, but we have been unable to determine its physical nature or even its precise location in this series of experiments Curiously, Hansson (1973) found a possibly similar barrier to diffusion to be present in the visual system extracellular HRP did not diffuse in either direction across the lamina cribrosa of the eye

Unlike Warr, we were successful in labeling the unmyelinated neurons of the spiral ganglion, but only in our animal perfused with ~63 μl of HRP which experienced the least cochlear trauma We do not believe at the present time that this uptake represents retrograde transport from terminals in the organ of Corti HRP perfused perilymphatically has ready access to the spiral ganglion, where we believe the unmyelinated neurons came into contact with the protein On the other hand Spoendlin (1971) has postulated that the unmyelinated neurons supply the outer hair cells with afferent fibers They could as easily be autonomic postganglionics as hypothesized by Ross & Burkel (1973) Ellison & Clark (1975) have shown that postganglionic neurons in viscera are labeled after local HRP injection

The facts that only ~0 063 mg of horseradish peroxidase labeled the unmyelinated neurons and, although possibly still deleterious to electrical activity, was least traumatic to the cochlea, suggest that lesser rather than greater concentrations of HRP may be appropriate for tracer studies in the inner ear This point was made early on with respect to capillary permeability investigations in general by Clementi (1970) as well Unfortunately the current trend in HRP work is to use high concentrations of this protein which in the case of the inner ear would appear to be unnecessary and even unrewarding in view of the doubtful validity of the data obtained

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ZUSAMMENFASSUNG

Die Cochlea von Meerschweinchen wurde *in vivo* mit einer

langsam wieder ausgeschieden Anzeichen akuter Zell schadigung waren Schwellung Vakuolisierung und diffuse Markierung einiger Haarzellen aber Stereocilia behielten normales Aussehen Albino Melanozyten im Ligamen tumspirale waren ebenfalls geschädigt und Vakuolisie rung der Reißnerschen Membran wurde bei 10%iger Kon zentration des Enzyms beobachtet Beide Konzentra tionen führten zur Erniedrigung des Mikrophonpotentials was eine akute Ototoxizitat von Meerrettichperoxidase anzeigt *Der Wirkungsmechanismus ist unbekannt* Retrograder Transport des Enzyms in Zellen des Spiralgang

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QUANTITATIVE ANALYSIS OF KANAMYCIN OTOTOXICOSIS

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Abstract The morphological changes after kanamycin intoxication of the inner ear including both the cochlea and the vestibule were quantitatively analysed by the surface preparation technique after succinic dehydrogenase staining 75 guinea pigs were used The outer hair cells in the basal coil and the inner hair cells in the upper coils of the cochlea were the most severely damaged but many unusual modes of damage were also revealed For example the initial hair cell damage in the cochlea appeared in the upper hair cells The clearly observed vestibular damage contradicts the general belief that kanamycin is not so toxic to the vestibular hair cells The utricular macula and the lateral crista were most severely damaged The delayed ototoxicity of kanamycin was observed for the first time in the vestibular hair cells

Numerous studies concerning the ototoxicity of kanamycin (KM) have been made and published since the earliest report by Frost et al 1959) The generally accepted concept about e characteristics of KM ototoxicity derived om both clinical and experimental studies is that the outer hair cells of the basal turn of the cochlea are more susceptible to KM intoxication However, many exceptions and individual differences have been observed

The purpose of this study is to observe quantitatively the cochlear and the vestibular hair cell damage caused by the surface preparation technique and to estimate general morphological features of KM ototoxicosis statistically by using a large number of animals

METHODS

Seventy five healthy albino guinea pigs weighing about 300 g were used These animals were divided equally into three groups (25 in each) which received daily intramuscular injections of KM (400 mg/kg body weight) during 7, 10 and 14 days, respectively The groups were named A, B and C The animals in each group were subdivided equally into five smaller groups according to the intervals until the histological examinations These intervals were 1 day, 1 week, 1 month, 3 months and 6 months after the last injection Symbols used to represent the smaller groups were 0, 1w 1m, 3m and 6m, and were added to the symbols of the larger groups, respectively For example, Group A1w means that the animals in this group were administered KM for 7 days (indicated by "A") and were sacrificed one week after (indicated by '1w) the last injection Likewise, Group 1m means that the animals of this group were sacrificed 1 month after the last injection including all the animals of Groups A, B and C Two animals d before the observation and one ear had faile during preparation of the specimen Therefore 145 ears from 73 animals were finall observed

The specimens were prepared as follow The animals were anesthetized in an ethe filled chamber and were then decapitated Th temporal ottic bullae were enucleated immed ately and the bony capsules opened The bon walls covering both the cochlear and the vest bular organs were carefully removed in saline and the membranous labyrinths then tran ferred into succinic dehydrogenase (SDH staining reagent, a mixture of 2 vol 0 1% nitro blue tetrazolium, 1 vol 0 2 M pH 7 4, phos

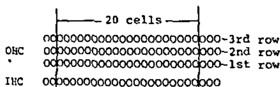


Fig 1 The surviving cell numbers in each row at the determined position of every coil in the cochlea were counted *OHC* outer hair cell, *IHC* inner hair cell

phate buffer and 1 vol 0 2 M sodium succinate They were bathed in water at 37 0°C constant temperature for 1 hour. After more than 24 hours of fixation in chilled 10% neutral formaldehyde, these specimens were prepared by the so-called "surface preparation technique"

The organ of Corti was separated from the modiolus, by removing the stria vascularis and the tectorial membrane under a stereoscopic microscope using a fine injection needle The dissected organ of Corti was embedded in glycerol on a glass slide The vestibular sensory epithelia, both of the macula and crista, were peeled from the subepithelial layer in the same way, and were flattened on glass slides with glycerol

When the specimen was observed from the upper surface under a light microscope, optical sections of any of the hair cell layers were selected The SDH reagent has the great advantage of being able to selectively visualize the sensory cells in any portion of the inner ear The hair cell damage can therefore be observed easily

Although the entire area of the organ of Corti was observed, from the apex to the hook end, only the surviving cells on the limited area at each coil were counted, for statistical convenience The degree of damage was displayed by the number of cells that survived in 20 cell positions in each row of the outer and inner hair cells, as shown in Fig 1

The hair cell degeneration of all vestibular organs was examined, lateral, anterior and posterior cristae, and utricular and saccular maculae Compared with the regular arrangement of the hair cells in the organ of Corti, the vestibular sensory cells were scattered throughout the network of supporting cells Thus, it was difficult to establish the exact number of hair cells that survived and the severity of vestibular damage was consequently evaluated by using a scoring method ranging from 0 (not affected) to 3 (most severely damaged) according to the standard grading as shown in Fig 2 The values of these scores were later used for the statistical analysis

RESULTS

The mean body weight changes of the KM administered animals measured on the 7th, 10th and 14th day of injections were markedly reduced, in contrast to those of the non-administrated animals (Fig 3) The dose of 400 mg KM per kg body weight was so excessive that it became difficult for their nutritional condition, though the damage to the hair cells was not caused by the poor nutritional state The specific patterns of hair cell degeneration in the inner ear, as shown in the following, revealed that this damage was caused by the specific ototoxic effects of KM, irrespective of the animals' general condition

Patterns of damage to the cochlear sensory hair cells

(1) The degree of the cochlear hair cell damage was closely related to the dose of KM The mean numbers of surviving outer hair cells in all parts of the cochleae (which were 80 in normal) were reduced to 65 7, 58 1 and 25 3 in the animals of Group A, Group B and Group C, respectively

Those of the inner hair cells were similarly reduced from 80 (normal) to 76 5, 70 8 and 33 4 in the animals of Groups A, B and C, respectively

The dose responses of the hair cell damage were thus established

(2) Table I shows the influence of the resting intervals after cessation of administration, upon the extent of cochlear damage

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QUANTITATIVE ANALYSIS OF KANAMYCIN OTOTOXICOSIS

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(Received November 25 1976)

Abstract The morphological changes after kanamycin intoxication of the inner ear including both the cochlea and the vestibule were quantitatively analysed by the surface preparation technique after succinic dehydrogenase staining 75 guinea pigs were used The outer hair cells in the basal coil and the inner hair cells in the upper coils of the cochlea were the most severely damaged but many unusual modes of damage were also revealed For example the initial hair cell damage in the cochlea appeared in the upper hair cells The clearly observed vestibular damage contradicts the general belief that kanamycin is not so toxic to the vestibular hair cells The utricular macula and the lateral crista were most severely damaged The delayed ototoxicity of kanamycin was observed for the first time in the vestibular hair cells

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The specimens were prepared as follows The animals were anesthetized in an ether filled chamber and were then decapitated The temporal ottic bullae were enucleated immediately and the bony capsules opened The bony walls covering both the cochlear and the vestibular organs were carefully removed in saline and the membranous labyrinths then transferred into succinic dehydrogenase (SDH) staining reagent, a mixture of 2 vol 0 1% nitro blue tetrazolium, 1 vol 0 2 M, pH 7 4, phos

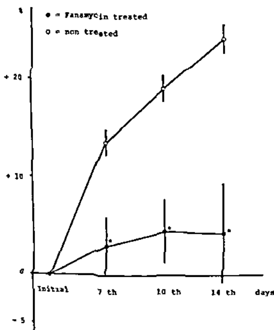


Fig 3 Mean body weights of 10 kanamycin-treated animals and those of 10 non treated animals on 7th 10th and 14th day after the initial injections The weight changes are shown by their percentage increases and standard errors Asterisks show the significantly decreased values compared with the simultaneous normal values

Type 0 specimens in which all the hair cells were damaged and had disappeared

The incidences of the types appearing in each group are summarized in Fig 4 Types 1 and 2 appeared most frequently in Group C, but conversely, Type 4 appeared rather more often in Group A This result suggested that Type 4 was the initial form of degeneration and Types 1 and 2 were the late form of damage, reaching the terminal form in Type 0 Types 3 and 5 were considered to be the intermediate forms, between Type 4 and Types 1 or 2 in the course of hair cell damage The reasons why the outer hair cells in the upper coils were more vulnerable to KM only at the initial stage of degeneration than those of the other parts of the cochlea will be discussed later

(4) The vulnerability of the three rows of outer hair cells differed from one to another and their degeneration patterns were characteristic in each coil As shown in Fig 5, the two inner rows were more vulnerable in the upper two coils of 3rd and 4th, whereas the *innermost row was more vulnerable in the 2nd* coil than in the others In contrast to these upper three coils, the outer hair cells in the basal coil were almost equally damaged at the three rows from the initiation of damage From 106 inner ears, except those of Type N and Type 0, the means and standard errors of the surviving hair cell numbers were calculated separately for each row in each coil of the cochleae The statistical significances were evaluated by *t* test

The values marked with an asterisk, were significantly smaller than the others in the same coil ($p<0.01$) No significant difference was observed between each pair of values of the inner hair cells in any coil combination

Patterns of damage to the vestibular sensory hair cells

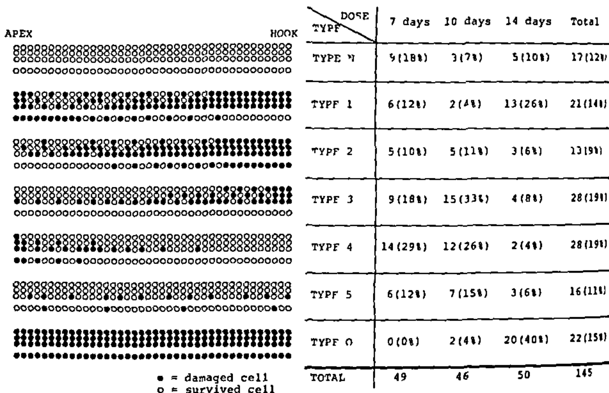
This work revealed that the vestibular damage caused by KM was not so slight and it was occasionally very extensive and even as profound as that caused by streptomycin The damage observed in the vestibular organ was considered to be specific for the KM administration because of its dose response

(1) Table II shows the influence of the resting intervals after KM administration upon the vestibular damage The mean scores of vestibular damage progressed steadily during the

Table I *Mean values and standard errors of the surviving hair cells in the whole cochleae are shown according to the resting intervals after the last injections*

OHC outer hair cell IHC inner hair cell

	OHC	IHC
Immed after	70 4±3 3	74 8±2 8
1 week after	45 6±5 1	64.5±5 3
1 month after	49 9±6 2	54 3±6 7
3 months after	42 9±5.3	55 9±5.5
6 months after	38 5±5 7	49 7±6 8



DOSE / TYPE	7 days	10 days	14 days	Total
TYPE N	9(18%)	3(7%)	5(10%)	17(12%)
TYPE 1	6(12%)	2(4%)	13(26%)	21(14%)
TYPE 2	5(10%)	5(11%)	3(6%)	13(9%)
TYPE 3	9(18%)	15(33%)	4(8%)	28(19%)
TYPE 4	14(29%)	12(26%)	2(4%)	28(19%)
TYPE 5	6(12%)	7(15%)	3(6%)	16(11%)
TYPE 0	0(0%)	2(4%)	20(40%)	22(15%)
TOTAL	49	46	50	145

Fig 4 Schematic patterns of the cochlear damage and their incidences

resting intervals, in all groups In order to elucidate this finding statistically, the animals that rested for identical intervals were combined to form each different administration group The means and standard errors of the stibular damage scores of all 145 inner ears re calculated, respectively, according to the sting intervals from 0 to 6 months after the ast injections (Table III) In comparison with the value of Group 1w, Group 0 was significantly spared from damage The values of Groups 1m, 3m and 6m increased, respectively, in accordance with the prolongation of the resting period The value of Group 6m reached a significantly higher level than Group 1w ($p<0.05$) While cochlear damage did not proceed during the resting intervals the vestibular damage did progress steadily during these periods

(2) Vestibular hair cell damage occurred almost simultaneously in both ears in the same individual The correlation of crista and macular damage between the ears on the two sides is shown in the scatter diagrams (Fig 6) Each dot in these diagrams represents the crista damage scores on both sides, which were calculated from the damage to the lateral, anterior and posterior cristae The macular damage represents the combined damage scores of both saccular and utricular maculae

The correlation coefficient was calculated by using the combined damage scores, including all the vestibular apparatuses in all the ears The coefficient was 0.894, which means a significant correlationship of vestibular damage between the two sides ($p<0.01$)

Table II *Mean scores of the vestibular damage in all groups*

Doses	Immed after	Observed after 1 week	1 mo	3 mo	6mo
7 days	0	0 89	0 10	0 20	1 30
10 days	0 30	0 50	2 20	3 13	2 50
14 days	0 20	4 00	5 80	6 80	7 40

```
                         4th Coil

      15 6±0 42    o o o o o o o o o o o o o o o o ● ● ● ●
OHC   16 8±0 44    o o o o o o o o o o o o o o o o o ● ● ●
      12 7±0 81*   o o o o o o o o o o o o o ● ● ● ● ● ● ●

IHC   16 2±0 69    o o o o o o o o o o o o o o o o ● ● ● ●

                         3rd Coil

      17 4±0 51    o o o o o o o o o o o o o o o o o ● ● ●
OHC   16 6±0 59    o o o o o o o o o o o o o o o o o ● ● ●
      11 5±0 91*   o o o o o o o o o o o o ● ● ● ● ● ● ● ●

IHC   17 5±0 61    o o o o o o o o o o o o o o o o o o ● ●

                         2nd Coil

      16 4±0 67    o o o o o o o o o o o o o o o ● ● ● ● ●
OHC   11 3±0 81*   o o o o o o o o o o o ● ● ● ● ● ● ● ● ●
      10 7±0 86*   o o o o o o o o o o o ● ● ● ● ● ● ● ● ●

IHC   18 2±0 50    o o o o o o o o o o o o o o o o o o ● ●

                         1st Coil

      11 6±0 86    o o o o o o o o o o o o ● ● ● ● ● ● ● ●
OHC   11 8±0 85    o o o o o o o o o o o o ● ● ● ● ● ● ● ●
      11 6±0 85    o o o o o o o o o o o o ● ● ● ● ● ● ● ●

IHC   17 5±0 60    o o o o o o o o o o o o o o o o o o ● ●

                                    o = surviving hair cell
                                    ● = damaged hair cell
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Fig 5 A comparison of the hair cell damage in each row in each coil The values show surviving cell numbers and standard errors The asterisks show significantly smaller values among the outer hair cells in the same coils ($n=210$ $p<0\ 01$)

(3) The vulnerability to KM of the various parts of the vestibular apparatus differed from one to another (Table IV) The utricular macula was more frequently and more severely damaged than the saccular macula ($p<0\ 01$)

The lateral crista was the most frequently and most severely damaged of the three kinds of cristae ($p<0\ 01$) The difference between the damage scores of the anterior and the posterior cristae was not significant

The vulnerability difference between the macula and the crista was difficult to assess, as the cytoarchitecture and the damage pattern in both sensory areas differed considerably As for the macula the sensory hair cells in the striola were initially and most strikingly damaged, while the hair cells other than those of the striola were much more resistant One damaged macula appeared as if only the hair cells in the striola had disappeared (Fig 7) In contrast to the macula, the damage to the crista was not so clear (Fig 8)

(4) The two types of the vestibular hair cells reported by Wersall (1969) as Type I and Type II cells were occasionally hard to evaluate on the surface specimen stained by SDH reaction and the vulnerability difference between the two types was also obscure However it was found in some well preserved specimens

Table III *Mean scores of the vestibular damage in all groups*

Asterisk shows the significantly larger value compared with that of 1 week after ($n=55$ $p<0\ 0?$)

	Means ±S E
Immed after	0.50±0 22
1 week after	1 83±0 38
1 month after	2 70±0.50
3 months after	3 46±0 78
6 months after	3 82±0 68*

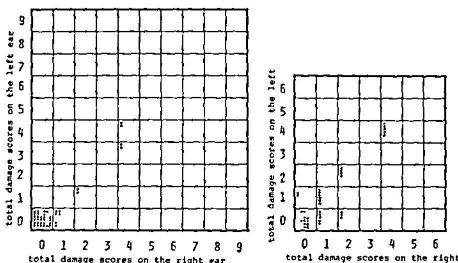


Fig 6 Comparison of the crista (*left*) and macular (*right*) damage between the ears on both sides

that Type I cells seemed to degenerate more readily than Type II cells as a result of KM intoxication

DISCUSSION

Since the discovery of KM by Umezawa (1958), numerous experimental studies on ototoxicity have been reported (Ward & Fernán dez, 1961, Beck & Krahl, 1962, Reddy & Igarashi, 1962, Farkashidy et al , 1963, Hawkins & Engstrom 1964, Engstrom & Kohonen, 1965, Kohonen 1965 and other reports) generally accepted concepts concerning ototoxic pattern seemed to be established owever accurate details have not been subjected to statistical evaluation, especially as regards the vestibular influence

Our method of evaluating cochlear hair cell damage was to calculate the number of damaged or restored hair cells within a limited area of each coil in the cochlea Although there is a minor disadvantage in not being able to recognize the overall damage to each cochlea, this method was found convenient to grasp the overall tendency of damage using statistical analysis The results obtained by this method were compared with some overall microscopic observations over the entire area of the organ of Corti and it was ascertained that they did not differ so much from the actual features

We have clinically experience of a few cases showing delayed hearing impairment after the cessation of administration of some kinds of basic aminoglucosidic antibiotics such as dehydrostreptomycin (DHSM) or neomycin as has been already reported by Hawkins (1959) and Kohonen (1965) However, delayed ototoxicity of KM has not been reported hitherto It has been supposed that KM ototoxicity does not progress after the cessation of administration It was ascertained in this series of experiments that the delayed ototoxicity of KM to the cochlear hair cells has not occurred On the contrary, the vestibular hair cells continued to be damaged even after the cessation of KM administration, although the delayed

Table IV *Mean scores of the damage in each vestibular organ*

The t value with asterisks indicate the significant difference between the matched two values ($n=288$ $p<0.01$) A lat Ampulla lateralis ant anterior post posterior

	Mean and S E		
Saccule	0 193±0 038	$t=7.909$*	
Utricle	0 972±0 091		
A lat	0 690±0 080	$t=2.985$*	$t=3.868$*
A ant	0 331±0 055	$t=0.927$	
A post	0 248±0 046		

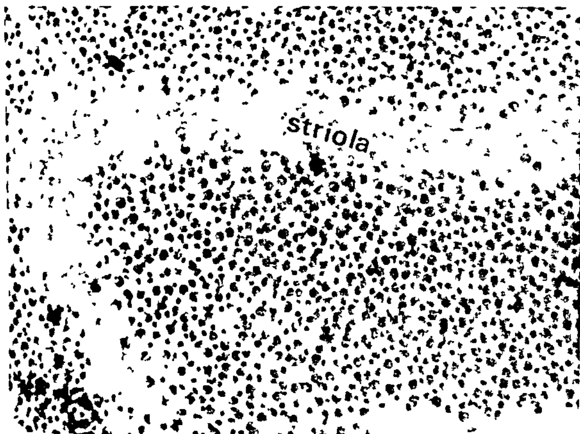


Fig 7 Localized striolar degeneration of the macula sacculi

ototoxicity of KM to the vestibular systems has not yet been clinically or experimentally determined The susceptibility difference between the cochlear and the vestibular hair cells after stopping KM appeared to be attributable to the structural differences between the two organs The cochlear hair cells especially the outer hair cells are floating in Nuel s lymphatic space whereas the vestibular hair cells are compactly surrounded by the adjacent supporting cells The existence of KM in the supporting cells seemed to be more prolonged than in the lymphatic fluid The delayed vestibular damage observed in this investigation gives rise to considerations as to its clinical use

It has been generally accepted that the outer hair cells of the basal coil are the most susceptible to KM poisoning Our data also confirm the great vulnerability of the basal outer hair cells in many specimens However there has been no reasonable explanation for this susceptibility Engstrom & Kohonen (1965) and Kohonen (1965) suggested that the form of the efferent nerve endings attached to the outer hair cells was responsible for the susceptibility of the hair cells and that the easily damaged hair cells possessed large granular nerve endings which were distributed mainly amongst the outer hair cells in the basal and especially at their innermost row The outer hair cells in the upper coils have been thought to be relatively resistant to KM although we found that the upper outer hair cells were occasionally destroyed to a much greater extent than those of the lower coils The specimens in which we found more extensive destruction of the cochlear upper part than the lower part

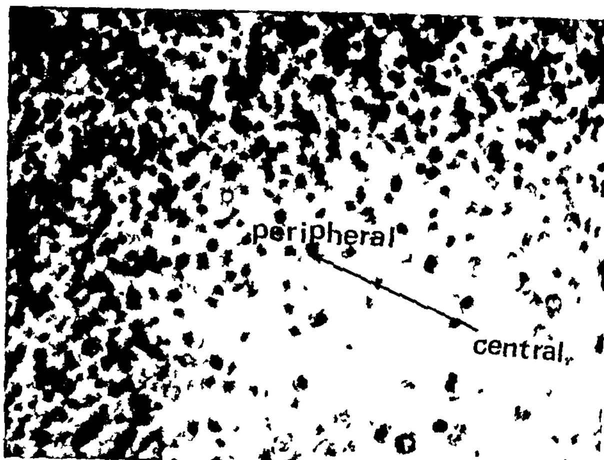


Fig 8 Diffuse central degeneration of the crista anterior

longed mostly to those of Group A We refore suggest that the initial hair cell damage seems to appear in the apical coil of the cochlea The hair cell destruction of the lower coil was delayed but it later became more extensive than the apical destruction

A few cases showing the early susceptibility of the upper outer hair cells have been reported by Kohonen & Tarkkanen (1969) However the drug used by them was neomycin which moreover was administered through the tympanic cavity of the guinea pig In their paper they concluded that the susceptibility of the upper coil may well be related to the upper directional flow of perilymph and the accumulation of the drug in the upper part However their results and comments are not so helpful for us in evaluating our results as their experimental procedure is so essentially different

Dieroff & Beck (1966) found that the protein storage in the upper coil of the cochlea decreased after chronic sound stimulation

Stockwell & Ades (1969) also revealed that the upper hair cells were easily damaged by lower frequency sound stimulation which seemed to inhibit the intra cellular protein synthesis As revealed by Spott & Stainer (1961) the basic aminoglucosidic antibiotics have an inhibitory potency against bacterial protein synthesis Although the action of KM on cellular protein synthesis is different from that on bacterial protein synthesis the above mentioned experimental results of Dieroff & Beck Stockwell & Ades and Spott & Stainer suggested that the damage to the upper hair cells

by KM might be attributable to the protein synthesis impairment. The conspicuous damage of the lower hair cells at the late stage appears to be correlated to the high intracellular aerobic respiration rate of the lower hair cells (Mizukoshi & Daly, 1967). The incorporation of KM is activated by the cellular aerobic respiration (Tanaka, 1960). Thus incorporated, the KM gradually accumulates in the lower hair cells and the highly concentrated KM later damages the lower hair cells more extensively than the upper hair cells. The time lag in the initiation of damage between the upper and the lower hair cells is explained by the time required for the accumulation.

From clinical experience, KM does not cause vestibular impairment, and vestibular damage caused by KM has been reported by only a few authors (Ward & Fernandez, 1961, Farkashidy et al., 1963, Lindemann, 1969, Watanuki, 1972 and Ogawa, 1975). Watanuki found mild sensory damage in the crista ampullares of the guinea pigs treated with KM, but he described that this was much milder than that in the cochlea. The other authors found no remarkable vestibular damage caused by KM. All of them concluded that the vestibular sensory cells were much more resistant to KM than the cochlear hair cells. The present work has revealed that even the vestibular hair cells were occasionally damaged by KM. The vestibular damage was often not so mild, especially when KM was given in larger amounts.

One of the reasons for the rarity of clinical incidence of vestibular damage following KM, compared with our experimental results, would seem to be the small size of the KM dose. Our results also showed that the vestibular damage was found mainly in those animals dosed with a larger amount of KM, although the vestibular hair cells of those dosed with a smaller amount of KM were almost free from damage.

This study has revealed that the hair cells of the utricular macula and the lateral crista are more apt to be damaged by KM than the other vestibular parts. This finding agreed closely with previous reports concerning the vestibular damage caused by streptomycin (Lindemann, 1969 and other reports). However, the reasons for such differences in vulnerability among each sensory region has not yet been clarified. By electron microscopic observation Smith (1970) has already revealed that the vestibular subepithelial layer is freely connected with the perilymphatic space, and that the substance in this space can relatively easily penetrate into the subepithelial layers. This suggested that KM can reach the vestibular sensory cells via the perilymph where the administered KM can easily accumulate (Stupp et al., 1967). The bottoms of the lateral crista and the utricular macula present a broad face to the perilymphatic space, while the other cristae and macula are buried in the narrow bony canal. This difference in the interrelationship between the sensory regions and the surrounding perilymph would seem to explain the vulnerability differences to KM among each sensory region.

ZUSAMMENFASSUNG

Die quantitative Analyse der morphologischen Veranderungen der Innenohrhaarzellen nach Kanamyzinvergiftung in der Schnecke und dem Vestibulum wurde durch Hautchenpraparationstechnik nach Succinodehydrogenasenfarbung beobachtet. Die Beschadigung der außeren Haarzellen in der Basalschnecke und der inneren Haarzellen in der oberen Schnecke war am starksten, aber viele außergewöhnliche Schadigungstypen wurden auch bemerkt. Die vestibulare Haarzellenschadigung, im Gegensatz zur allgemeinen Ansicht, namlich daß Kanamyzin nicht so toxisch gegenuber den vestibularen Haarzellen sei, wurde auch deutlich beobachtet. In den fünf vestibularen Apparaten waren die Utriculusmacula und die Lateralkrista am schwersten beschadigt. Die verspatete Ototoxizitat von Kanamycin zu vestibularen Haarzellen wurde 6 Monate nach der nachsten Injektion beobachtet.

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SLOW EVOKED CORTICAL RESPONSES TO LINEAR FREQUENCY RAMPS IN SUBJECTS WITH COCHLEAR LESIONS

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Abstract Slow evoked cortical potentials in response to linear frequency ramps of a continuous pure tone with a 1 kHz base frequency have been recorded from ten relatively young subjects with hearing loss of cochlear origin At small frequency ramps the N_1 latency of their responses to the three ramp durations studied (20 100 500 msec) was significantly longer than those of a group with normal hearing As the ramps are made larger the difference between the latencies of the pathological group and the normal group becomes smaller above a certain rate of frequency change the latency of N_1 becomes smaller in the pathological cases than in the normal group This crossover occurs at frequency change rates around 1–3 kHz/sec It is concluded that recording of evoked cortical responses to frequency ramps may provide an additional tool in the differential diagnosis of hearing disorders

Auditory frequency discrimination has been studied extensively by means of various types of stimuli and different psychoacoustic methods Most of these experiments have been concerned with determination of the threshold for frequency change i e difference limen for frequency, in subjects with normal hearing (e g Shower & Biddulph 1931 Harris, 1952, Konig, 1957, Sergeant & Harris 1962 Moore, 1973) In some studies frequency discrimination has been investigated in subjects with various types of hearing loss (e g Butler & Albrite, 1956, Meurmann, 1954 Parker et al , 1968) Several studies have shown that slow cortical potentials evoked in response to tones with frequency ramps can be recorded from the scalp (Spoor et al , 1969, Ruhm, 1970, Lenhardt, 1971) These studies have been confined to normal hearing subjects

Behavioral thresholds have been determined for linear frequency ramps of a continuous pure tone in a group of normal subjects and compared with those from a group with hearing loss of cochlear origin (Arlinger et al , 1976*a*, *c*) Slow evoked cortical responses to the same type of frequency ramps have been recorded from the same normal group (Arlinger et al , 1976*b*) In the present study, similar scalp-recorded cortical potentials from the group of subjects with cochlear hearing loss are studied and compared with those of the normal group Recordings of cortical responses from subjects with retrocochlear lesions will be reported later

The amplitude and latencies of the slow evoked cortical response have been shown to vary with the stimulus i e its intensity or rate of frequency change, etc It has, for example, been shown that the response amplitude gradually increases as the intensity of tone burst stimulation is increased, while response latencies gradually decrease It may thus be possible to use evoked responses in a quantitative evaluation of supra threshold stimulation, and conclusions about the auditory mechanisms involved may be drawn that are not immediately evident from threshold studies

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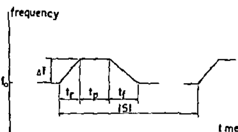


Fig 1 The temporal characteristics of the stimulus

SUBJECTS

The same 10 young subjects – mean age 30 years – as were examined previously in the study of behavioral thresholds for frequency change (Arlinger et al , 1976*c*), participated in this study (for further audiometric test results and other details on the subjects, see Arlinger et al , 1976*c*) The subjects had hearing losses of cochlear origin according to conventional audiometric test results

Before each session of electrophysiological recording the behavioral threshold was determined for the same stimulus subsequently used to evoke cortical responses (the stimulus proper had different magnitudes of frequency change) Each electrophysiological session began with recordings of responses to standard stimulus in order to monitor each ject s performance from session to session to compare inter and intra subject varia on

STIMULUS

The stimulus consisted of a continuous pure tone of 1 kHz base frequency f_0 (see Fig 1) The frequency of the tone was changed linearly The inter stimulus intervals (ISI) were varied randomly from 2 sec upwards with a mean interval of 4 sec The linear frequency sweep, regarded as the actual stimulus was characterized by its magnitude (Δf) and its duration (t_r) After the end of each sweep the signal remained at the final frequency of the sweep during t_p before returning to the base frequency during t_f The sum of t_r and t_p was kept constant at 500 msec t_f was always 600 msec All harmonics of the signal were 40 dB or more below the fundamental as acoustically measured in a 6 cc coupler

The stimulus was always presented at each subject's most comfortable level which ranged from 60 to 90 dB HL, the mean for the 10 subjects was 75 dB This corresponded to sensation levels in the 11–46 dB range with a mean of 28 dB

For a more detailed description of the stimulus generator and the response recording equipment, see Arlinger et al (1976*b*)

The ongoing cortical electrical activity picked up by Ag/AgCl electrodes on vertex and ear lobes was amplified, low pass filtered (cut off frequency of 12 Hz, attenuation 36 dB/octave) and fed into a digital averager Fifty responses were summed with a sweep time of 500 msec for 200 address points The accuracy of the latency determinations was thus 2 5 msec as read from the digital printout Response amplitude was read from the recorded averaged response

EXPERIMENTAL PROCEDURE

During the experiments, the subject was seated in a sound insulated anechoic chamber and monitored by intercom and TV The stimulus was presented monaurally, using a *Telephonics* TDH 39 ear phone fitted with a circumaural cushion All subjects read material of their own choice during the test

Each session began with a standard test employing constant stimulus parameters (t_r= 10 msec, Δf=50 Hz)

After the standard test, the actual test was performed Three frequency ramp durations (t_r) were studied – 20 100 and 500 msec – for upward sweep (increasing frequency) The three durations were assigned to the three test sessions in random order for each subject

During each test session, the average cortical responses to frequency ramps of 10 20 50 100 200 and 500 Hz were recorded in random order The total time required for each session was 25–30 minutes

able I *Means of individual means, standard deviation of individual means and mean of individual standard deviations for* N_1- *and* P_2-*latencies and* N_1P_2-*amplitude of the responses to the standard stimulus*

	Mean of individ means (ms or μV)	S D of individ means		Mean of individ S D s	
		ms or μV	%	ms or μV	%
N_1	130	15 7	12 2	8 1	6 4
P_2	228	15 0	6 6	12 8	5 6
N_1P_2	12 3	3 6	29	3 4	28

RESULTS

The mean values and standard deviations of N_1 and P_2 latencies and N_1P_2 amplitude in the averaged responses to the standard stimulus are presented in Table I (The N_1 is the vertex negative component found in the latency range of 100–200 msec, P_2 is the vertex positive component in the latency range 200–300 msec)

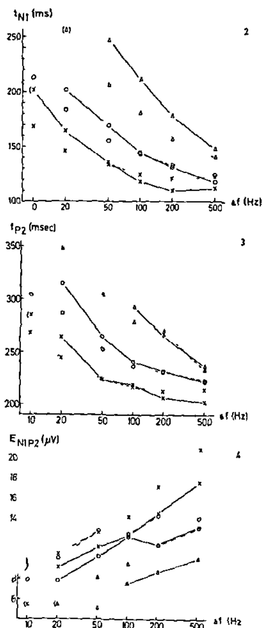
The mean values of the latencies of the two components are somewhat greater in this group than in the group with normal hearing (Arlinger et al , 1976*b*) The difference in N_1 latency (130 versus 125 msec), however, is not statistically significant The difference in P_2-latency (228 versus 216 msec) is significant on the 5%-level (Student's t test) The N_1P_2-amplitude is somewhat smaller for the coch lear group than that previously reported for the normal group (12 3 versus 13 9 μV), but the difference is not significant

No significant rank correlation exists ($p>10\%$) between any of these response characteristics and the behavioral thresholds the standard stimulus in this group of ten subjects nor was any significant rank correla tion found between the individual standard deviations of the response parameters

Figs 2, 3, and 4 show the mean values of N_1 latency, P_2 latency and N_1P_2-amplitude as functions of Δf with ramp duration as a para meter for the pathological group (solid lines) Also shown are the corresponding mean alues for the normal group (dashed lines) r small frequency ramps, the cochlearly impaired group generally showed longer N_1- and P_2-latencies than did the normal group For larger frequency ramps and short ramp duration, i e at large rates of frequency change, the latencies are shorter in the cochlearly impaired group than in the normal group, thus the relation is reversed compared with the responses to small frequency ramps Furthermore, no identifiable response could be obtained in the cochlear group for the smallest ramps at ramp durations of 100 and 500 msec (i e at the lowest rate of frequency change) Table II shows the levels of significance for the differences between the results from the two groups The sign * at some of the levels indicates that fewer subjects in the pathological group than in the normal group produced identifiable responses Thus the real difference in these cases may be greater than that shown by the level of significance For example, at $t_r=20$ msec and $\Delta f=10$ Hz, only 3 of the cochlearly impaired subjects produced a clear response, while all 10 normal subjects did

In spite of this, the N_1 latencies are significantly greater for the cochlearly impaired than for the normal group at Δf-values less than 50 Hz for $t_r=20$ msec, less than 100 Hz for $t_r=100$ msec and for all Δf values studied at $t_r=500$ msec The N_1 latencies in the cochlearly-impaired group are significantly shorter than those in the normal group at large frequency sweeps of $\Delta f>50$ Hz for $t_r=20$ msec

The differences in the mean values of the P_2-latencies between the two groups are generally not statistically significant The fact that the standard deviations for the P_2-



Figs 2–4 The mean N_1 latencies (Fig 2) P_2-latencies (Fig 3) and N_1P_2 amplitudes (Fig 4) as functions of frequency deviation for ramp durations of 20 msec (×) 100 msec (O) and 500 msec (Δ) Results from the cochlearly impaired group are connected by solid lines and those from the normal group by dashed lines The symbol within brackets indicates that responses could not be obtained from all 10 subjects in the group

latencies are larger than those for the N_1 latencies may explain this finding The difference in latency between the N_1 and P_2 components of the responses in the cochlearly impaired group shows no consistent pattern of change when Δf and t_r are changed The me[an] difference is 95 msec with a standard deviati[on] of 8 6 msec, which is not significantly differe[nt] from the result in the normal group The me[an] N_1P_2 amplitudes in the cochlear group a[re] smaller, though not always significantly s[o] than in the normal group for all values of [Δf] and t_r

DISCUSSION

The latencies of the responses to the standa[rd] stimulus show a smaller intra subject tha[n] inter subject variation particularly for the N component The variations in respons[e] amplitude on the other hand are of the sam[e] magnitude This is at variance with the find[-] ings in normal subjects (Arlinger et al 1976b) where the two variations were of essentiall[y] equal magnitude or differed slightly in the op[-] posite direction Generally the normal grou[p] showed smaller variations, particularly be[-] tween subjects than the cochlearly impaire[d] group This may be explained by the patho[-] logical group s being less homogeneous tha[n] the normal one, as indicated by the large[r] inter subject variation in their behavioral thresholds (38 versus 23 %)

As in subjects with normal hearing there was no significant rank correlation between any of the three electrophysiological response characteristics evoked by the standard stimulus and the behavioral thresholds in the hearing impaired subjects

At other values of Δf and t_r the responses of the cochlearly impaired group show some interesting and clear differences compared to those of the normal group In the pathological group N_1 latencies significantly longer than those of the normal group were obtained for small frequency sweeps at ramp durations of 20 and 100 msec and for all frequency sweeps studied at 500 msec ramp duration (Fig 2 and Table II) This indicates that e g a 10 or 20 Hz frequency change with a ramp duration of 20 msec constitutes a weaker stimulus for the subjects with cochlear hearing

Table II *Levels of significance (Student's t test) for differences in latencies and amplitudes between results from cochlear and normal groups NS = not significant difference*

* Indicates that differences may be more significant than levels given (see text for explanation)

Δf (Hz)		10	20	50	100	200	500
t_{N1} at t^r =	20	5%*	2 5%*	NS	5%	2 5%	2.5%
	100	–*	2 5%*	2 5%	NS	NS	NS
	500	–	–*	0 1%*	0 5%*	0 05%	10%
t_{P2} at t^r	20	NS*	NS*	NS	NS	NS	5%
	100	–*	5%*	NS	NS	NS	NS
	500	–	–*	–*	NS*	NS	NS
NS							
$E_{N,P}$ at t^r =	20	2 5%*	NS*	NS	NS	10%	10%
	100	–*	2 5%*	5%	NS	2 5%	NS
	500	–	–*	0 05%*	10%*	2 5%	1%

loss than for the normal subjects This agrees well qualitatively with the elevated behavioral thresholds found in the cochlearly impaired as compared to the normal group (mean thresh olds on the average 2 8 times higher) (Arlinger et al , 1976*c*)

In response to large frequency sweeps and 20 msec ramp duration, the cochlearly impaired group produced significantly shorter N_1 latencies than the normal group A similar tendency can be seen for the other ramp durations This finding can be analysed further by studying the general course of t_{N1} as a function of Δf or $s = \Delta f / t_r$ as in the following model This mathematical model was found to describe accurately the data from the normal group

$$t_{N1} = t_0 + t_1 \; (s/s_0)\beta \qquad \text{eq 1}$$

and

$$t_{N1} = t_0 + t_2 \; (\Delta f / \Delta f_0)\beta \qquad \text{eq 2}$$

where t_0 is chosen to give the best fit between computed and experimental results within the studied range of Δf and t_r, t_0 is the latency which t_{N1} is assumed to approach for very large stimuli, t_1 and t_2 represent the differences in latency between very large stimuli and a chosen reference slope s (kHz/sec) and a reference sweep Δf_0 respectively β is a negative constant

This model describes the data from the cochlear group with the same accuracy as it did that for the normal group (evaluated by means of linear regression analysis of the logarithm of the equations)

The value of t_0 giving the best fit for a given ramp duration (determined by a maximum of the squared correlation coefficient) is significantly lower ($p < 1\%$) for the cochlearly-impaired group than for the normal group (mean values 86 versus 110 msec) This indicates an extension towards larger frequency ramps of the experimental data shown in Fig 2 In other words, the N_1-latency relations may be reversed for all ramp durations, and not only for the 20 msec duration verified by the experimental results The frequency sweep where the N_1-latencies become equal for the two groups was found to have a higher magnitude when the ramp duration was increased This crossover occurs at rates of frequency change ($\Delta f / t_r$) in the range of 1 to 3 kHz/sec, where the latencies obtained are 130–140 msec

No basic qualitative difference between the normal and the pathological groups was found when the relationship between the N_1 latencies and the rate of frequency change was compared This finding agrees with the results of the behavioral threshold studies in both groups (Arlinger et al , 1976*a c*), and supports the previous conclusion that the magnitude of the frequency sweep, Δf, is the crucial

property of the stimulus in evoking the cortical response to small ramps of short duration With larger ramps and ramp durations above the range 20–100 msec (depending on the rate of frequency change), the evoked cortical response becomes almost exclusively dependent on the rate of frequency change, $\Delta f/t_r$ These results fit the functional model proposed earlier (Arlinger et al , 1976*a*, *b*) for the auditory response to frequency sweeps This model involves integration of the time derivative of the signal frequency with an integration time which is a function of the rate of frequency change The most pronounced quantitative difference between the results obtained in the two groups is that in the N_1-latencies Small frequency sweeps evoke significantly longer N_1-latencies in the cochlearly-impaired group, agreeing with this group's increased behavioral thresholds, while large frequency sweeps evoke significantly shorter N_1-latencies in this group

The perceptual implication of these findings is worthy of note If there is a perceptual correlate to these electrophysiological results one might postulate a "recruitment of pitch change" in connection with cochlear impairment analogous to the well known "recruitment of loudness" The existence of this phenomenon, though not yet shown directly, may be considered to be supported by preliminary results of a test using a binaural balance method on subjects with unilateral cochlear hearing loss

The possibility that such a perceptual correlate may exist is further supported by the findings that the N_1 latencies are qualitatively related to perception at near threshold stimulation, reflecting the decreased sensitivity of the pathological group in detecting frequency ramps Also normal subjects exhibit a qualitative relation between the influence exerted by different base frequencies (250–4 000 Hz) and different sound levels (20–80 dB HL) on thresholds for frequency change and on the N_1-latencies (Arlinger et al , 1976*b*) Furthermore, the N_1-latencies of the averaged cortical response to tone pulses of different sound levels (constant rise time and duration) are related to the loudness of the tone pulses in normal subjects as well as in subjects with cochlear lesions with recruitment (Arlinger, 1975)

The N_1-latency reached a saturation within the ranges of Δf and t_r used, while the N_1P_2-amplitude did not Response amplitude and response latency are likely to reflect different properties of the activity in the neural pathways, consequently, saturation may occur in the latter without simultaneously occurring in the former

A frequency change in a continuous signal results almost inevitably in some simultaneous loudness change In two subjects with normal hearing, it was shown that controlled sound level changes in the range −5 to +5 dB, occurring simultaneously with a 200 Hz frequency change, had no significant influence on the evoked responses Such a state of affairs is likely to hold true in the cochlearly impaired group as well The subjects in the cochlear group were selected to have pure tone audiograms of a rather flat shape in the 1kHz region (pure tone thresholds at 0 5 and 2 kHz differing from that at 1 kHz by 10 dB or less) Subjects with cochlear lesions involving recruitment of loudness are known to have a lower difference limen for intensity at low sensation levels than subjects with normal hearing (Jerger et al , 1959) At higher loudness levels, the differences are supposedly negligible (Luscher, 1955, and the authors unpublished observations using intensity ramps) Since the stimuli used in the present study were kept at the most comfortable level, thus well above threshold, the DL is probably no different from that of the normal group Therefore, it is reasonable to assume that possible loudness changes can be neglected when comparing frequency changes in response generation in the cochlear group as was shown to be the case in the normal group

CONCLUSIONS

Slow evoked cortical responses to frequency ramps have been studied in 10 young subjects with hearing loss of cochlear origin and compared with their behavioral thresholds for the same stimuli These results are compared with those obtained in normal subjects The following conclusions can be drawn

(1) The N_1 latencies for small frequency sweeps were significantly longer in the pathological group than in the normal group This agrees with the results obtained when studying behavioral thresholds

(2) For larger sweeps the difference in N_1 latency between the normal and cochlearly impaired groups was found to decrease for all ramp durations studied The latencies became equal when the frequency sweeps corresponded to sweep rates in the range 1–3 kHz/sec Above this range the N_1 latencies of the cochlearly impaired group became significantly shorter than the normal latencies

(3) These results fit the previously proposed functional model for the perception of frequency ramps

(4) The recording of slow evoked cortical responses to frequency sweeps is indicated as providing an additional tool in the differential diagnosis of hearing disorders

ZUSAMMENFASSUNG

Die corticale Spatantworten auf uberschwellige Reize in Form lineare Frequenzrampen eines Dauertons (1 kHz) sind in 10 jungen Versuchsperson mit Innenohrschwerhörigke t untersucht worden Die N_1 Latenz dieser Gruppe war bei kleinen Frequenzrampen signifikant langer als die einer Normalgruppe Bei grosseren Frequenzrampen war aber kein Latenzunterschied zu sehen und bei den grossten Rampen mit kurzer Dauer (hochster Frequenzänderungsgeschwindigkeit) war die N Latenz dieser pathologischen Gruppe sogar kurzer als die einer Normalgruppe Es wird zusammengefasst dass Registrierung corticalen Spätantworten auf Frequenzrampen eine brauchbare d agnostische Methode werden mochte

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THE INFLUENCE OF PERILYMPHATIC PRESSURE ON THE DISPLACEMENT OF THE TYMPANIC MEMBRANE

A Quantitative Study on Human Temporal Bones

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Abstract A model study on human temporal bones was performed A microflow method was used in order to assess the possibility of making indirect recordings of changes in the inner ear pressures Changes in the perilymphatic pressure were recorded as displacements of the tympanic membrane the stapedius reflex being artificially elicited by forces applied briefly to the stapedius tendon Changes in the perilymphatic pressures in the range of +15 cm H_2O affected the position of the tympanic membrane and the stapedius reflex response The microflow method used seems suitable for the clinical record ng of changes in the intralabyrinthine pressure

report exists in clinical work on a reli e indirect method by which changes in the ner ear pressure can be recorded In animal experiments however the inner ear pressure has been studied directly after opening the labyrinth (Martinez 1969, Moscovitch et al 1973 Berndt et al 1975) The present report may be considered as part of a series of studies on the middle ear/inner ear pressure relationship (Densert et al 1975 Ivarsson & Pedersen 1976 Cassel brant et al 1976 Ingelstedt et al 1976) Politzer (1861) and Bezold (1908) have already shown using temporal bones that volume displacement of perilymph as caused by overpressure applied to the external ear canal or in the middle ear, can be observed directly via a fistula in the semicircular canal In animal experiments Kobrak (1935) showed a relationship between middle ear pressure and labyrinthine fluid displacement Studies on temporal bones were later pe formed quantitatively by Ivarsson & Pede sen (1976) The present report deals with th reverse problem i e whether a variatio in the perilymphatic pressure can be mea ured on the outside of the tympanic mem brane

In some clinical experiments attempts hav been made to measure inner ear pressur variations indirectly, e g by changing th body position from sitting to lying (Corsc 1962, Miltich, 1968 Macrae, 1972), or b neck vein compression (Klockhoff et al 1966) These procedures are followed by ar increasing venous pressure and an increasin CSF pressure which is transmitted to th inner ear These authors found either a lowering of the auditory threshold or a change in the impedance of the tympanic membrane which they interpreted as a sig of increased inner ear pressure

In the present report we explored the possibilities of indirect recording of changes in the inner ear pressure on the human temporal bone The degree of volume dis

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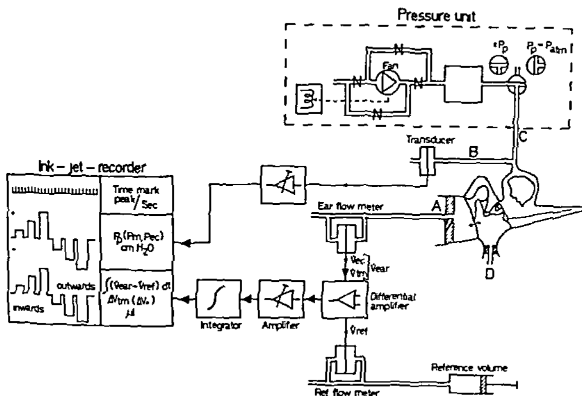


Fig 1 The volume flow and pressure equipment

placement caused by the tympanic membrane was recorded when various tensions were applied to the tendon of the stapedius at varying perilymphatic pressures

MATERIAL AND METHODS

Five human temporal bones were used, taken from patients with an average age of 47 years (range 23–65) In order to be used in the experiments the selected subjects had to fulfil the following criteria (i) no previous ear disease or neurological disorder as judged from the patient's case history and autopsy report, (ii) normal tympanic membrane and middle ear at otomicroscopic examination

Experiments were performed less than 24 hours after death During the interval between autopsy and experiments the temporal bones were kept at +5° in a humid chamber

The equipment consisted of a microflow method (Elner et al, 1971), which is illustrated in Fig 1 The flowmeter (ear flow meter) was connected to the ear canal (A) After amplifying and integrating the flow signal it was possible to record the volume displacement caused by the tympanic membrane (ΔV_{tm}) In order to record this volume displacement correctly, even at small variations in the ambient pressure and temperature, the equipment was fitted with an identical flowmeter (reference flow meter) connected to an adjustable reference volume When there was a balance in volume of the two flow systems, the isolated flow, caused only by the movement of the tympanic membrane, was recorded The sensitivity of the flow amplifier could be adjusted so that an integrated air flow volume of 0 005 μl through the flowmeter produced deflection of 1 mm on the ink jet recorder

A special pressure unit was connected hermetically to a fistula on the superior semi-

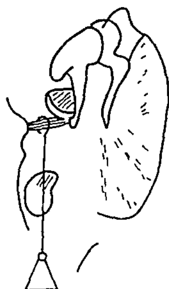


Fig 2 Arrangements for exerting tension on the stapedius tendon

circular canal (C) and the pressure it produced was recorded with a transducer at B For further calibrating data see Elner et al (1971), Ivarsson & Pedersen (1976)

Symbols used in the text and figures

P_{atm} atmospheric pressure at ground level
P_m middle ear pressure
P_p perilymphatic pressure
V_{tm} volume displacement caused by the tympanic membrane
Δ preceding a symbol means a change of the variable

Experimental procedure

The temporal bone was mounted in a fixed position with a vertical distance of about 1 5 cm between the fistula on the superior semicircular canal and the oval and round windows A polyethylene tube (C in Fig 1) was connected hermetically to the fistula The seal was checked in the pressure range of ±20 cm H_2O The flowmeter at A (Fig 1 ear flow meter) was connected to the external ear canal The volume displacement of the tympanic membrane was measured (ΔV_{tm}) as a function of the perilymphatic pressure (P_p) (C in Fig 1 and the middle ear pressure (P_m) (D in Fig 1)

Further, in order to record the volume displacement caused by the tympanic membrane the stapedius tendon was artificially pulled in an attempt to mimic the stapedius reflex contraction In order to reach the stapedius tendon the middle ear was opened via the fossa of the jugular vein A nylon thread was hooked around the tendon and loaded with weights of 0 6, 1 3, 1 6 and 2 6 g (Fig 2) The weight was applied to the tendon perpendicularly to the long axis of the tendon During recordings the nylon thread hung freely and did not come into contact with the walls of the temporal bone

RESULTS

1 The volume displacement caused by the tympanic membrane as a function of the middle ear pressure

This relationship is called the compliance of the tympanic system and refers to the mobility of the tympanic membrane of the ossicular chain, the elasticity of ligaments and the middle ear muscles This investigation was done to ensure that the elastic properties of the tympanic membrane system in the temporal bones were normal The mobility of the tympanic system in relation to the middle ear pressure is illustrated in Table I The results agreed well with those obtained

Table I *The compliance of the tympanic membrane system as a function of the middle ear pressure*

Middle ear pressure (ΔP_m cm H_2O)	Volume displacement caused by the tympanic membrane (ΔV_{tm} µl)				
	Case 1	Case 2	Case 3	Case 4	Case 5
+15	+24 0	+17 5	+14 0	+12 5	+17 0
+10	+22 0	+11 0	+12 5	+11 0	+10 5
+ 5	+18 0	+ 8 0	+ 8 5	+ 8 0	+ 7 5
+ 2 5	+12 5	+ 5 5	+ 4 5	+ 5 5	+ 3 5
0	0	0	0	0	0
− 2 5	− 7 5	− 4 5	− 3 0	− 5 0	− 5 0
− 5	−11 0	− 6 0	− 5 0	−11 0	− 7 0
−10	−15 0	− 9 0	− 7 0	−15 5	− 9 5
15	−17 5	−10 5	− 8 5	−18 0	−11 0

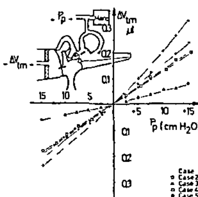


Fig 3 The volume displacement of the tympanic membrane (ΔV_{tm} in μl) as a function of the perilymphatic pressure (P_p in cm H_2O)

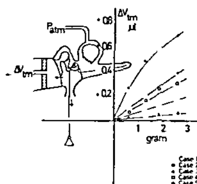


Fig 4 The volume displacement of the tympanic membrane (ΔV_{tm}) as a function of the weight (in grams) applied to the stapedius tendon

from previous investigations in humans performed by Elner et al (1971)

2 *The volume displacement caused by the tympanic membrane as a function of the perilymphatic pressure*

During these recordings atmospheric pressure was maintained in the middle ear Over- and underpressures in the range of ± 15 cm H_2O were applied to the perilymph Changes in the pressure of the perilymph affected the stapes and its movements were transferred to the tympanic membrane via the ossicular chain As shown in Fig 3, the volume/pressure relationship was almost linear in the investigated pressure range Only in case 3 the recorded gas volumes diverged from the others However, the middle ear structures were normal and mobile at microscopic examination

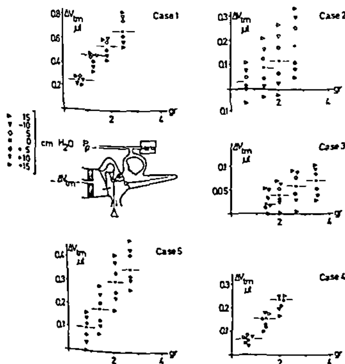


Fig 5 The volume displacement of the tympanic membrane (ΔV_{tm}) as a function of the force applied to the stapedius tendon (in grams) and of varied perilymphatic pressure (solid symbols = over pressure unfilled symbols = underpressure)

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AZIDOCILLIN AND AMPICILLIN CONCENTRATIONS IN MIDDLE EAR EFFUSION

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Abstract The penetration of azidocillin and ampicillin into the middle ear effusion after oral administration was studied and compared with blood levels of the drugs In acute otitis media one hour after a single oral dose of azidocillin (15 mg/kg) 1.56±0 44 μg/ml of the drug was found in the effusion fluid after 2 hours the fluid contained 3 21±0 87 μg/ml and after 12 hours 0 84±0 13 μg/ml The concentrations of ampicillin were 1 15±0 23 μg/ml after one hour, 2 17±0 46 μg/ml after 2 hours and 1 09±0 22 μg/ml after 8 hours following a single oral dose of 10 mg/kg Both drugs stayed longer in the middle ear secretion than in the blood This persistence supports the administration of azidocillin twice daily in acute otitis media In contrast the penetration of the drugs into the middle ear effusion fluid was poor in secretory otitis media where the levels of the drugs were significantly lower

The commonest etiological agents of acute otitis media at present are pneumococci and *Haemophilus influenzae* (Bergholtz & Rudberg, 1972, Feingold et al , 1966, Grönroos et al , 1964, Kamme et al , 1971) Infections due to β hemolytic streptococci and staphylococci have markedly diminished

Azidocillin (Globacillin®) has an antibacterial spectrum similar to that of penicillin G (Forsgren, 1968) It is slightly less active than ampicillin against strains of *H influenzae* (Tunevall, 1973) Against pneumococci, however, their activities are about equal (Tunevall & Frisk, 1967) The treatment of acute otitis media with azidocillin given twice daily has been successful (Bergholtz et al , 1973, Magnuson, 1973)

In the blood, 83–85% of azidocillin is bound to serum proteins (Sjoberg et al , 1967) Among the penicillins, ampicillin is the least affected in this way, 82% being present as free antibiotic (Rolinson & Sutherland, 1965) Although this binding is essentially a reversible one it is one of the important determinants of the drug distribution in the body Highly bound drugs tend to remain in the intravascular compartment, giving high blood levels, whereas lightly bound drugs diffuse more rapidly into the interstitial fluid (Kunin, 1965)

During recent years increasing attention has been paid to so-called secretory otitis media (Kokko, 1974, Palva, 1975, Palva et al , 1975, Senturia, 1963) Although the pathogenesis of this condition has not been completely solved (Brockman, 1972, Shapiro, 1975), the inadequate administration of antibiotics has been claimed to be a contributing factor in its prevalence (Kokko, 1974, Palva, 1975)

The purpose of the present work was to study in both acute and secretory otitis media the concentrations of ampicillin and azidocillin in blood and in middle ear effusion fluid

MATERIAL AND METHODS

The material consisted of 101 ambulatory patients with acute otitis media and 63 patients with secretory otitis media The patients had not received any previous antimicrobial treatment for their current illness In the present

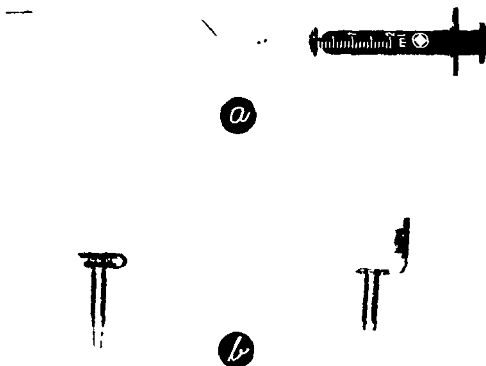


Fig 1 (*a*) Aspiration needle (*b*) Micro-centrifuge tube

study secretory otitis means an otitis in which the main symptoms are blocking of the ear nd impaired hearing for 3–7 days The tym ic membrane was neither reddened nor lging The aspirated fluid was serous or seromucous The cases of acute otitis media were characterized by an acute onset with severe pain The tympanic membrane was inflamed and sometimes bulging The as pirated fluid was purulent and often tinted with blood

Ear samples were taken by aspiration of the punctured middle ear cavity (Punctio cavi tympani) by means of Lahikainen s needle (Lahikainen 1953) and a plastic syringe (Fig 1*a*) The volume and quality of the aspirate were recorded The samples tinged with fresh blood were excluded The aspirated fluid was expelled through the needle into a small dis posable micro centrifuge tube (Fig 1*b*) and sent off immediately to the National Public Health Laboratory in Turku, where the drug concentrations were determined by micro-technique (Jalling et al , 1972) The samples were concurrently cultured on blood agar and McLeod agar plates The organisms isolated were identified by the usual methods and their drug sensitivities were determined The deter minations were carried out on PSA-agar (Difco) using Sarcina lutea ATCC 9341 as test organism The standard series for the respec tive antibiotics were obtained from Biodisk AB

The blood samples of children were taken from the finger tip by means of heparinised capillary tubes Ten μl of blood was trans ferred to a paper disc The blood samples of adults were taken by venous puncture The determination of antibiotics was performed as described for the aspirated middle ear effusion

Table I *Concentrations of azidocillin (Globacillin®) in middle ear effusion and in blood after oral administration of 15 mg/kg bodyweight of azidocillin to patients with acute otitis media or secretory otitis media*

	Middle ear effusion (μg/ml)			Blood (μg/ml)		
	1 hour	2 hours	12 hours	1 hour	2 hours	12 hours
Acute otitis media						
Number	20	15	17	10	14	14
Mean	1 56	3 21	0 84	9 82	7 37	0 04
Range	0 37–7 80	0.58–14 0	0 25–1 75	4 0–22 0	1 7–27 0	0–0 25
S E	0 44	0 87	0 13	1 76	1 83	0 02
Secretory otitis media						
Number	15	18	13	10	15	8
Mean	0 22	0.50	0 56	9 67	4 82	0 03
Range	0–0 50	0–1 60	0 2–1 20	2 0–25 0	1 2–12 0	0–0 20
S E	0 04	0 11	0 07	2 45	0 86	0 03

Seventy-eight patients with an average age of 23 3±2 6 (range 1–76) years were treated with ampicillin (Doctacillin®, Astra Lakemedel, Sodertälje, Sweden) and 86 patients with an average age of 28 9±2 2 (range 4–72) years with azidocillin (Globacillin®, Astra) The preparations—azidocillin at 15 mg/kg of bodyweight, and ampicillin at 10 mg/kg of bodyweight—were given orally

The samples were taken one, 2 or 8 hours after the administration of ampicillin, and one, 2 or 12 hours after administration of azidocillin Samples were taken immediately after the first dose on the first day of the treatment A total of 115 samples of ear secretion (63 samples for ampicillin and 52 samples for azidocillin determinations) and 82 blood samples (44 for ampicillin and 38 for azidocillin determinations) were taken from the patients with acute otitis media From the patients with secretory otitis media 74 secretion samples (28 samples for ampicillin and 46 samples for azidocillin determinations) and 56 blood samples (23 for ampicillin and 33 for azido cillin) were obtained

Total albumin, IgA, IgG, IgM and lysozyme concentrations of the aspirated fluid were also determined These results will be reported separately

Table II *Concentrations of ampicillin (Doctacillin®) in middle ear effusion and in blood after oral administration of 10 mg/kg bodyweight of ampicillin to patients with acute otitis media or secretory otitis media*

	Middle ear effusion (μg/ml)			Blood (μg/ml)		
	1 hour	2 hours	8 hours	1 hour	2 hours	8 hours
Acute otitis media						
Number	31	15	17	21	12	11
Mean	1 15	2 17	1 09	4.34	4.30	0 2
Range	0.2–6 4	0 85–8 0	0.25–3 25	0.3–11.5	1 0–9 0	0–0 6
S E	0 23	0 46	0.22	0 74	0 75	0 07
Secretory otitis media						
Number	13	9	6	9	9	5
Mean	0 17	0.23	0.57	3.25	3.62	0 08
Range	0–0.3	0–0.39	0.25–1 0	1.5–5.6	1 1–6 6	0–0 4
S E	0 03	0 05	0 14	0 41	0 60	0 08

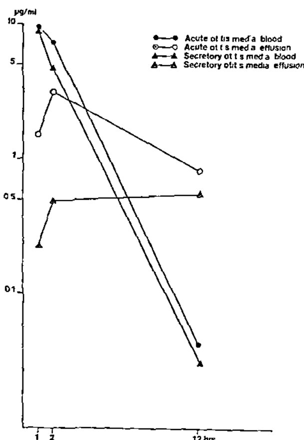


Fig 2 Concentrations of azidocillin in blood and in middle ear effusion fluid

RESULTS

The results are shown in Tables I and II, and in Figs 2 and 3

zidocillin was rapidly absorbed, giving blood concentrations in both groups The eak level, one hour after administration of the drug was 9 82±1 76 µg/ml in acute and 9 67±2 45 µg/ml in secretory otitis media After 12 hours only traces of azidocillin, 0 04±0 02 µg/ml and 0 03±0 03 µg/ml respectively were found

After one hour 1 56±0 44 µg/ml and after 2 hours 3 21±0 87 µg/ml of azidocillin were found in the ear secretions in acute otitis media Azidocillin stays longer in the middle ear effusion than in the blood After 12 hours a considerable amount of azidocillin 0 84±0 13 µg/ml (range 0 25–1 75 µg/ml) was still found in the ear secretion, so that the concentration was at this time approximately 20 times higher than that in blood The concentration was 0 25 µg/ml only in two cases The others showed a concentration of 0 40 µg/ml or more Bacterial cultures from ear secretions were negative except once when pneumococci were cultured one hour after the administration of azidocillin

The concentrations of azidocillin in secretory otitis media were lower After one hour only 0 22±0 04 µg/ml of the drug was found in the middle ear effusion fluid Compared with the acute otitis patients the difference is statistically significant (P<0 01) After 2 hours 0 50±0 11 µg/ml of azidocillin was found and here the difference is also statistically significant (P<0 01) Also, in the secretory otitis the level of azidocillin was sustained longer in the ear secretion than in the blood After 12 hours 0 56±0 07 µg/ml of the drug was still detected Bacterial cultures were negative except once when *H influenzae* was cultured one hour after the beginning of the treatment

Ampicillin gave lower blood levels than azidocillin After one hour 4 34±0 74 µg/ml and after 2 hours 4 30±0 75 µg/ml of ampicillin was found in the blood of patients with acute otitis media In the secretory otitis the concentration was 3 25±0 41 µg/ml after one hour and 3 62±0 60 µg/ml after 2 hours After 8 hours the blood levels had dropped in both groups, only 0 2±0 07 µg/ml and 0 08±0 08 µg/ml of ampicillin were found The concentration of ampicillin in the middle ear secretion was clearly lower in secretory otitis After one hour 1 15±0 23 µg/ml of the drug was found in the acute otitis and only 0 17±0 03 µg/ml in the secretory otitis The difference is statistically highly significant (P<0 001) After 2 hours the concentrations were 2 17±0 46 µg/ml and 0 23±0 05 µg/ml The difference is statistically highly significant (P<0 001)

The concentration of ampicillin was sustained longer in middle ear secretions than in blood After 8 hours 1 09±0 22 µg/ml of ampicillin was found in the patients with acute

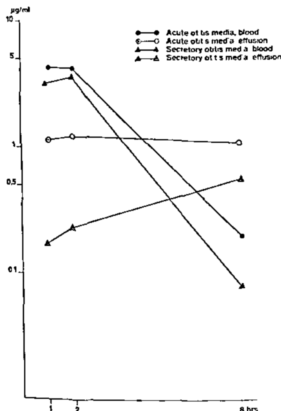


Fig 3 Concentrations of ampicillin in blood and in middle ear effusion fluid

otitis and 0 57±0 14 µg/ml in the patients with secretory otitis media All bacterial cultures from ear secretions were negative in secretory otitis *S aureus* was cultured twice in the acute otitis patients and *H influenzae* once one hour after the administration of ampicillin

The ratios of blood level to middle ear secretion level were for ampicillin 3 77 after one hour and 1 52 after 2 hours in acute otitis media In the secretory otitis the ratios were 19 12 after one hour and 15 74 after 2 hours

The corresponding ratios for azidocillin were 6 29 and 2 30 in acute otitis In the secretory otitis the ratio was 43 95 after one hour and 9 64 after 2 hours

DISCUSSION

The concentrations of ampicillin and azidocillin in the middle ear effusion fluid are high enough to be effective against β hemolytic streptococci and pneumococci (Sjöberg et al, 1967) About 90% of *H influenzae* strains are inhibited by 0 32 µg/ml of azidocillin and by 0 32 µg/ml of ampicillin (Forsgren, 1969, Kamme, 1969) The concentrations of the drugs obtained here in acute otitis media are sufficient for *H influenzae* and though the levels in secretory otitis are too low for this organism in the beginning they might be effective in prolonged use

The concentrations of ampicillin and azidocillin in the middle ear effusion fluid increase more slowly than in blood, but are sustained longer The same has been observed earlier with penicillin G (Lahikainen, 1970) The sustained levels of drugs in middle ear secretions may be caused by their relative dissociation from the normal inactivating and eliminating processes taking place in the body The sustained level of azidocillin in the middle ear secretion after 12 hours seems to call for the administration only twice a day The clinical results of treatment with azidocillin administered twice daily were satisfactory (Bergholtz et al, 1973) With ampicillin the level in middle ear effusion fluid was still high after 8 hours There is so far no experience of 12 hours dosage of ampicillin

The penetration of ampicillin and azidocillin into the middle ear secretion was poor in secretory otitis This is probably associated with the mechanism of the secretion In secretory otitis media a local production of the effusion is assumed However whatever the etiology and pathogenesis of secretory otitis may turn out to be, the chances of successful therapy using these agents are considerably less promising for the poor penetration of drugs

The ratios of blood level to middle ear secretion level in acute otitis media were smaller for ampicillin than for azidocillin Ampicillin which is less bound to serum proteins is clearly more rapidly transferred into the middle ear secretion

The bactericidal effect of the drugs was

surprisingly rapid. After one hour the cultures were positive in only 4 cases. All the subsequent cultures were negative

The blood level of the drugs correlates rather poorly with the level in the middle ear effusion fluid for the different penetrations of the drug in two forms of otitis media and for the sustained presence of the drugs in the effusion.

ZUSAMMENFASSUNG

In dieser Untersuchung wurden die Konzentrationen von Azidocillin und Ampicillin im Mittelohrsekret bestimmt und mit entsprechenden Blutkonzentrationen verglichen Bei akuter Otitis media betrug die Azidocillinkonzentration im Mittelohrsekret eine Stunde nach einmaliger oraler Zufuhr von 15 mg/kg Azidocillin 1,56±0,44 μg/ml, zwei Stunden nach der Dosierung 3,21±0,87 μg/ml und zwölf Stunden nach der Applikation 0,84±0,13 μg/ml Die entsprechenden Werte nach einmaliger oraler Zufuhr von Ampicillin beliefen sich auf 1,15±0,23 μg/ml, 2,17±0,46 μg/ml und nach 8 Stunden 1,09±0,22 μg/ml Bei beiden Mitteln wurde die Konzentration im Mittelohrsekret länger aufrechterhalten als im Blut Dieser Befund spricht dafür, daß bei akuter Otitis media Azidocillin zweimal [illegible]

entsprechend signifikant geringer

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ENZYMATIC ACTIVITY OF INNER AND MIDDLE EAR FLUIDS IN FETAL GUINEA PIGS UNDER AMBIENT AND HIGH-INTENSITY SOUND PRESENTATION[1]

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Abstract The enzymes in perilymph of the inner ear scala and within fluid filling the middle ear cavity were investigated in the fetal guinea pig near term Perilymph lactic dehydrogenase (LDH) exhibited differential activity between fetal scalae vestibuli and tympani and LDH aldolase phosphohexose isomerase and creatine phosphokinase in perilymph of particular fetal scalae were elevated in activity in comparison to corresponding maternal values Moreover LDH displayed different isozyme patterns for antenatal and gravid perilymph In the middle ear fluid all of the above enzymes were encountered but varied somewhat from the quantities determined for either prenatal perilymph or maternal serum After gravid guinea pigs were exposed to a tone of 4 kHz 100 dB SPL for 2 hours LDH of the fetal middle ear fluid was reduced in activity 24 hours later The metabolic significance of the fetal distinctions in the ear fluids and the clinical implications of the current work are discussed

The biochemical nature of the inner ear fluids from elementary electrolytes to complex macromolecules, has been elucidated in recent years Our knowledge, however is primarily based upon specimens taken from mature animals of different species The idea that the biochemical composition of the fluids may not necessarily be the same throughout development has not been tested for even a single species The present study represents initial efforts toward investigating such a possibility The objective was to examine the enzymes in the labyrinthine fluids of the guinea pig during one of the earliest stages of maturation, namely the fetal period, and to uncover distinctions between fetal and maternal samples Moreover, some specimens were procured after gravid animals were exposed to intense acoustic stimulation This procedure was instituted in order to determine the extent to which the biochemistry of fetal and gravid fluids is susceptible to such environmental treatment Since the middle ear cavity of the fetus is completely filled with a clear, colorless or sometimes slightly yellowish fluid, such mixtures were included in the biochemical analyses and the findings compared with those of other fetal as well as maternal fluids

MATERIALS AND METHODS

Forty-four gravid guinea pigs weighing between 720 and 1 690 g and displaying a Preyer's reflex were employed as subjects Females near term were randomly assigned to either an experimental or a control group where the former comprised 10 animals The experimental treatment consisted of exposure to

[1] Presented in part before the Biochemical Section of the Tenth Great Lakes Regional Meeting of the American Chemical Society Evanston Illinois June 17–19 1976

Table I *Enzymatic activities of fluids from maternal and fetal guinea pigs exposed to ambient or high-intensity sound* [a,b]

	Sound exposure	Ambient condition			
Fluid	LDH (mU/ml)	LDH (mU/ml)	ALD (mU/ml)	PHI (U/l)	CPK (U/l)
Maternal					
Serum	194 5±50 0	240 4±22 2	25 1±1 7	1 022 9±33 7	129 2±13 5
Perilymph					
Scala vestibuli	105 7±20 6	58 2± 8 9	6 6±0 8	82 8± 7 9	15 4± 2 6
Scala tympani	122 5±15 2	76 6± 6 0	6 9±0 8	78 8± 4 6	36 6± 8 2
Fetal					
Middle ear	122 5±27 1	352 2±28 7	6 7±0 6	73 8±12 6	24 1± 6 8
Perilymph					
Scala vestibuli	119 4± 9 2	147 0±15 0	10 6±0 8	169 5±10 4	34 7± 3 3
Scala tympani	94 9±10 3	79 6±12 5	8 1±0 4	158 5± 9 7	22 2± 2.5

[a] For LDH the number of samples (N) amounted to 8 and for all other enzymes $N=10$ Each value is the mean ± standard error

[b] Tone of 4 kHz, 100 dB SPL applied for 2 hours and fluids collected 24 hours after the treatment The background intensity measured 30 dB SPL

sound stimulation at 4 kHz and 100 dB SPL for 2 hours about 24 hours prior to collection of samples The acoustic instrumentation and its arrangement have previously been described (Gershbein et al , 1974) The background intensity of the control or ambient condition measured 30 dB SPL

Blood was obtained from the gravid animals under ether by cardiac puncture The fetuses were removed by cesarean section and a litter of two to seven was provided in each stance They measured 10–13 cm from wn to rump and weighed 65–135 g The ditory bullae of gravid and fetal pigs were leared of surrounding tissue and then opened to expose the middle and inner ears In the case of the fetus samples of fluid filling the middle ear cavity were obtained for biochemical analyses Prior to collection of inner ear fluids from fetal and maternal sources, the areas adjoining the oval and round windows were thoroughly dried Perilymph was drawn from the scalae vestibuli and tympani of the cochleae as previously described (Gershbein et al , 1974) except that the present procedure did not damage or remove any middle ear or vestibular structures All specimens were centrifuged at 3 000 rpm for 10 minutes and subsequently examined for the presence of erythrocytes Any visibly contaminated sample was not included in the enzymatic determinations

The activities of lactic dehydrogenase (LDH), aldolase (ALD) and phosphohexose isomerase (PHI) in the different fluids were determined by kinetic assay, use also being made of Calbiochem and Worthington kits In addition, the LDH isozyme distribution in the fluids was ascertained by polyacrylamide disc electrophoresis The above procedures have been described in a previous report (Gershbein et al , 1974) The present creatine phosphokinase (CPK) assay is based on a series of coupled reactions resulting in NADH The reduction of one μmole NAD per minute at 30°C is taken as one international unit (U) CPK activity, measured at 340 nm, is expressed as U/l

RESULTS

The results of the biochemical and electrophoretic analyses of fluids from fetal and maternal sources are presented in terms of means and standard errors in Tables I and II Prior to statistical analysis, values in the form of percentage were transformed to degrees

Table II *LDH isozyme percentages of maternal and fetal guinea pig fluids*[a]

Fluid	LDH isozyme 1	2	3	4	5
Ambient conditions (intensity 30 dB SPL)					
Maternal					
Serum	34 4±7 0	19 8±3 0	27 9±4 3	16 8±1 5	1 1±0 7
Perilymph					
Scala vestibuli	60 9±4 3	22 8±1 7	12 7±2 3	3 6±0 9	-
Scala tympani	55 3±4 6	23 8±2 4	15 8±2 0	5 1±1 4	-
Fetal					
Middle ear	23 5±6 2	16 3±1 9	28 3+3 4	28 0±3 6	4 0±1 3
Perilymph					
Scala vestibuli	34 4±4 4	22 5±3 5	20 2±1 8	16 6±2 3	6 4±2 2
Scala tympani	28 9±3.3	26 4±2 5	25 7±1 4	16 6±2 1	2 4+1 0
Sound exposure (tone of 4 kHz 100 dB SPL)					
Maternal					
Serum	26 4±3 8	19 6±1 5	30 3±4 4	21 1±2 9	2 7±2 0
Perilymph					
Scala vestibuli	65 4±4 7	21 1±2 0	10 3±2 5	3 2±1 2	-
Scala tympani	47 0±3 9	27 9+4 4	14 6±2 4	7 6±2 7	3 0+1 6
Fetal					
Middle ear	17 6±2 3	19 3+1 8	34 8±2 4	24 0±3 2	4 2±2 2
Perilymph					
Scala vestibuli	27 3±1 8	20 8±2 3	25 4±2 7	20 7±1 7	5 8±1 5
Scala tympani	26 5±2 4	22 9±2 2	28 7±2 1	19 1±1 8	2 9±1 3

[a] All means ± standard error are based on 8 samples

by use of the inverse sine function (Snedecor, 1946) Analysis of variance (Edwards, 1968) was employed to ascertain the statistical significance of the data Wherever a significant F ratio was obtained, the multiple comparison method of Newman and Keuls (Winer, 1962) was also applied to determine the significant pairs among a set of means

Of the enzymes examined in antenatal inner ear fluids only LDH (P<0 01) exhibited differential activity according to perilymph scala, namely, the scala vestibuli presenting a greater activity than that of the scala tympani With respect to the isozymes of LDH only LDH_5 showed a disparity between scalae where it too was greater in the scala vestibuli (P<0 05) Comparison of perilymph from fetal and maternal sources yielded a number of significant differences Specifically, in contrast to adult perilymph prenatal samples displayed greater LDH (P<0 01) ALD (P<0 01) PHI (P<0 01) and CPK (P<0 05) activity in the scala vestibuli and higher PHI activity (P<0 01) in the scala tympani In terms of percentages of LDH isozymes, antenatal perilymph was less in LDH_1 (P<0 01) and greater in LDH_3 (P< 0 05), LDH_4 (P<0 01) and LDH_5 (P<0 01) in the scala vestibuli and lower in LDH_1 and more in LDH_3 and LDH_4 (in each case, P< 0 01) in the scala tympani than was its maternal counterpart

The fluid filling the middle ear cavity of the fetus exhibited significant differences in enzymatic activity in contrast to the other fluids In comparison to maternal serum, it showed elevated activity in LDH (P<0 01) and decreased ALD, PHI and CPK (in all cases, P<0 01) Also, this fluid presented lower ALD activity than prenatal vestibular perilymph (P<0 05) and higher LDH (P<0 01) and lower PHI (P<0 01) relative to antenatal perilymph of either scala Similarities in enzymatic activity were observed for ALD in fetal middle ear fluid and fetal tympanic perilymph (P>

0 05) and also for CPK in the middle ear fluid and fetal perilymph from both scalae ($P>$ 0 05) In regard to the LDH isozymes, the mid dle ear fluid possessed more LDH_4 than in the case of maternal serum or prenatal perilymph from either scala (in each, $P<$0 05)

Finally, in relation to experimental treatment, acoustic stimulation led to a decrease in total LDH of the fetal middle ear fluid ($P<$0 01) and to the exclusion of any effect on the LDH activity of the other fluids ($P>$0 05) or on the isozyme patterns of any of the products (either $P>$0 05 or $F<$1 00)

DISCUSSION

The present investigation characterizes sev eral facets of the biochemical nature of the inner ear fluids during the earliest stages of development In the fetus, perilymph appears to possess enzymatic activity which varies, in at least one instance, according to region with in the cochlea For example, among the en zymes LDH, ALD, PHI and CPK found in antenatal perilymph, the first parameter showed greater activity in the scala vestibuli as compared to the scala tympani In addition, more LDH_5 occurred in the scala vestibuli than in the scala tympani Presently, the sig nificance of these findings is not certain Per ps the dissimilarities reflect a functional dif entiation between the scalae

A comparison of inner ear fluids drawn from maternal animals and their fetuses was con ducted in order to infer possible metabolic alterations during maturation Several enzy matic differences between prenatal and adult labyrinthine fluids were observed and from these data pertinent metabolic aspects are clarified One revelation is that the metabolic rate in perilymph appears for the most part to be greater during the fetal stage than in adulthood, in keeping with the general rapid growth during gestation Other insight into the metabolic processes of the inner ear fluids is gained by examination i the LDH isozyme patterns since LDH_1 an l l DH_5 are known to be correlated with aerobic and anaerob metabolism, respectively (Dawson et al 1964) Accordingly, the LDH isozyme dis tributions in both fetal and maternal perilymph indicate a prevalence of aerobic metabolism but with the fetal pattern tending toward greater anaerobiasis The latter is in keeping with the lowered oxygen availability and the heightened glycolytic activity of the fetus

The observation that fluid fills the middle ear cavity of the fetus prompted a study de signed to elucidate its composition The re sults reveal that this fluid is endowed with enzymatic activity which is unique in some respects and similar in other ways to mater nal serum and neighboring fetal perilymp' The lack of a close biochemical correspon ence among the fluids indicates that the midc ear liquor is not directly derived from eith fetal perilymph or maternal serum The fin ings do not preclude the possibility of an i direct relationship with the implication some type of intermediate ultrafiltratio However, a more reasonable possibility is th the middle ear product originates from tl amniotic fluid Since the latter is swallowe by the fetus in utero (Becker et al 1940) might flow from the fetal oral cavity throug the nasopharynx and the Eustachian tubes in the middle ear cavity Whether such a hyp thesis is tenable might be evaluated by a cor parison of biochemical parameters for th middle ear and amniotic fluids work of whi is currently in progress Other approache might include administering dyes or isotop ically-labeled substances into the amniotic sa and tracking their possible passage into th middle ear cavity Such experiments might b extended toward the screening of benefici and detrimental effects of drugs injected sys tematically into the gravid animal or directl through the amnion upon the fetal auditor mechanism The approach might allow for th analysis of fluid constituents such as enzyme by amniocentesis (Ressler 1974) for the as sessment of auditory conditions prior to birth

The elucidation of the biochemical natur

of the fetal ear fluids is a prerequisite for subsequent investigation of the extent to which the composition of these media can be influenced by experimental treatments. Previous studies have shown that the fetus does react in utero to sound from the environment. For example, acoustic stimulation elicits changes in the electroencephalogram (Scibetta & Rosen, 1969), the electrocardiogram (Grimwade et al., 1970, Tanaka & Arayama, 1969) and the body movement (Tanaka & Arayama, 1969) of the fetus. In the current investigation the effects of intense sound were ascertained on the total LDH and isozyme levels of the fetal ear fluids. Although no significant action could be demonstrated in the case of perilymph, a decrement in total enzyme of the middle ear fluid was observed. The decrease was not related to any specific isozyme but was associated with a general decline in the activity of all isozymes. In the absence of additional data, an explanation for the enzymatic depression would be purely conjectural at this time. Perhaps the effect is attributable to sound acting directly on the fetus, to the intermediate intervention of maternal factors or to both. An answer might be inferred from the screening of neural, endocrinological and cardiovascular parameters of the adult female and fetus in addition to delineation of the effect of sound on the other enzymes. The present study is of an exploratory nature and additional work is currently under way involving the determination of electrolytes, glucose, protein and other glycolytic enzymes in the fetal labyrinthine fluids, including endolymph, over a wide developmental range up to term and employing additional animal species as such and exposed to acoustic treatment.

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ZUSAMMENFASSUNG

Die Enzyme der Perilymphe aus der Scala vestibuli, der Scala tympani und in der Flüssigkeit der fötalen Mittelohrenhöhle wurden in späten trächtigen Meerschweinchen untersucht. Die Lactatdehydrogenase (LDH) der Perilymphe zeigte besondere Aktivitäten zwischen den Scalae vestibuli und tympani. LDH, Aldolase, phosphohexose Isomerase und Kreatine Phosphokinase waren höher in Aktivität in der Perilymphe von besonderen fötalen Scalae im Vergleich mit den entsprechenden mütterlichen Werten. Außerdem waren die LDH Isoenzymmuster für die fötalen und mütterlichen Perilymphen verändert. In der Flüssigkeit des Mittelohres waren die Enzyme wie schon erwähnt vorhanden, aber sie wichen von den Mengen in fötalem und mütterlichem Blutserum ab. Nach dem trächtige Meerschweinchen zu einem Ton von 4 kHz 100 dB SPL für 2 Stunden ausgestellt wurden, war die LDH Aktivität der Mittelohrenflüssigkeit nach 24 Stunden vermindert. Die metabolische Bedeutung von fötalen Unterschieden in den Ohrenflüssigkeiten und die klinische Wichtigkeit dieser Arbeit werden behandelt.

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A NOTE ON DEVELOPMENT OF CORTI'S ORGAN

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Abstract The structure and postnatal transformation of Kolliker s organ in the cat were studied by means of both light and electron microscopes Kolliker s organ which is located in the inner sulcus area of the cochlear duct during embryonic development of the kitten appeared to undergo a major transformation during the first 30 days after birth On the level of the organ as a whole the possible nature of this transformation is briefly discussed On the cellular level the transformation of this organ was found to involve a process of cellular autophagocytosis Kolliker s organ consists of numerous tightly packed tall columnar cells filling the inner sulcus Autophagic vacuoles containing cell organelles were observed in these cells in all stages of transformation of the organ Cellular autophagocytosis reduced the number of cells present in each section from approximately 50 in the newborn to approximately 12 in the 30-day-old kitten The apparent transformation of Kolliker s organ was observed as progressing from base to apex and from the limbus to the inner hair cell The relationship of the tectorial membrane to Kolliker s organ and that of the tectorial membrane to the area in and around the inner hair cells are discussed

Developmental studies of the foetal organ of Corti have shown a striking configuration of tall-columnar cells filling the inner sulcus This structure was first described by Kolliker (1863) and referred to by Hensen (1863) as Kolliker s organ This organ has been observed in a variety of species including cattle and horses (Hensen 1863) man (Bast & Anson, 1949) mice (Kikuchi & Hilding, 1965, Sher, 1971) rabbits (Anggård 1965) and in cats, dogs and guinea pigs (Pujol & Marty, 1970, Pujol & Hilding 1973) Hensen (1863) pointed out that it was seen only in embryonic stages and that the tall-columnar cells were eventually replaced by cuboidal cells He observed that many of the columnar cells were lost but he was unable to determine their fate The various stages of the changes which occurred to this organ were illustrated by Boettcher (1869) In man this replacement process is complete in the 220 mm, 25 week-old fetus (Bast & Anson, 1949) Similarly, in the species mentioned in the above studies, the replacement process occurs during the embryonic and/or early postnatal stages of the animal s growth However, the nature of this process and the fate of the replaced columnar cells remains unknown Implicit in this problem is the question of the origin of the cuboidal cells replacing Kolliker's organ Hensen (1863) seemed to indicate that the former originate from the latter, an opinion supported by Pujol & Hilding (1973)

The purpose of our study was to follow as closely as possible, by means of both light and electron microscopy, the nature of the replacement of these columnar cells Specifically, we focused on the histological changes associated with this postnatal cell replacement occurring in Kolliker's organ in kittens

MATERIALS AND METHODS

The observations by light microscopy were made in a group of 50 kittens ranging in age

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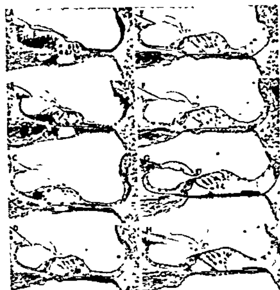


Fig 1. Postnatal development of Corti's organ in the cat. Each photomicrograph is of the basal turn. (A) Premature (B) 1 day (C) 5 days. (D) 10 days (E) 15 days. (F) 25 days (G) 30 days. (H) 60 days

from 4 hours to 30 days. The kittens were anesthetized with sodium pentobarbital (30 mg/kg) and perfused intravitally with Heidenhain-Susa fixative. Routine histological procedures were used for processing the temporal bones, cutting and staining serial sections (20 μm thickness) For electron microscopy studies, a group of 26 kittens ranging from 3 hours to 30 days old were used The cochlea were fixed intravitally and the specimens were prepared for electron microscopy as described elsewhere (Hinojosa, 1971).

RESULTS

Light microscopy

Some features of Kölliker's organ are depicted in Fig 1. A premature kitten (Fig 1*A*) showed a striking configuration of densely packed columnar cells lining the inner spiral sulcus from base to apex. The cells measured about 65 μm in height and about 3–4 μm in diameter In the 20 μm thick sections the nuclei appeared as described by Hensen (1863)—in two or three superimposed rows The tectorial mem-

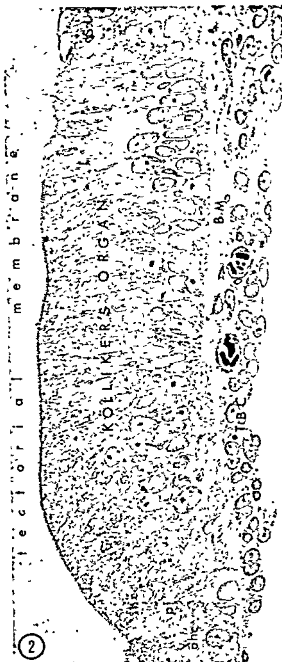


Fig 2 Low-power electron micrograph of Kölliker's organ (apical turn) of a premature kitten 4 hours after birth, showing the relation of this organ to the inner hair cell (*ihc*), spiral limbus (*SL*), tectorial membrane and basilar membrane (*BM*) ×672

brane of the premature kitten, unlike that of the full-term newborn kitten, was found in close contact with Kolliker's organ. In som



Fig 3 A port on of Koll ke ... n h ... ng a group of tall columnar cell The ... n a n nume ous m tochondria (*m*) ves ula ... and a nucleus (*n*) l cated at the basal end of the ... 6 6

sections there appeared to be an attachment by means of a filamentous structure

In the full term newborn kitten the con figuration of Kolliker s organ was quite d f ferent (Fig 1*B*) Throughout the cochlea the cells lining the medial aspect of the inner sul cus had been replaced by cuboidal cells (about 10 μm in height) so that the sulcus was only partially visible The tectorial membrane had been released but still appeared in close con tact with the remaining Kolliker s organ The transformation from columnar to cuboidal cells occurring within the inner sulcus epi thelium proceeded in orderly fashion from base to apex and from limbus to inner hair cells and appeared complete in about 4 weeks (Fig 1*B–H*) The loss of columnar cells was apparently considerable since in a section of 20 μm thickness over 100 cells of Kolliker s organ were replaced by about 12 cuboidal cells As in Hensen s study (1863) we d d not find it possible to follow an actual process of apparent replacement of columnar cells by cuboidal cells However definite s gns of cytoplasmic change were seen in certain cells as evidenced by the appearance of vacuoles containing fragmented material No macro phages were observed

Electron microscopy

Electron micrographs of Kolliker s organ fill ing the inner spiral sulcus showed about 50–55 tightly packed cells (Fig 2) The cells were arranged in long columns about 70 μm in height and 5 μm in diameter (Fig 3) their apical plasmalemma showing numerous tall and thick microvilli (Fig 4) Occas onal cells had a small kinocilium The junctional com plex between cells generally showed a zonula occludens and a zonula adherens (Fig 6) In about 30% of the junctions desmosomes in various stages of development were found (Figs 7 8)

The intercellular spaces measured about 200 Å throughout the length of the lateral plasmalemma some gaps of about 30 Å were found The nuclei were irregular in shape and

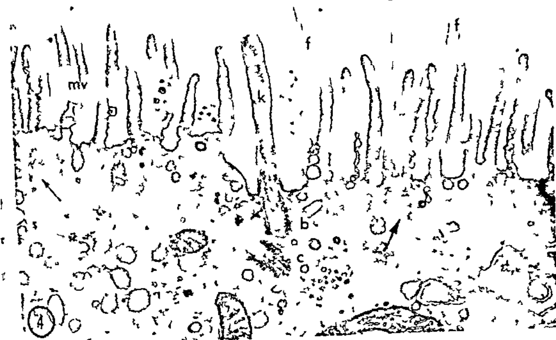


Fig 4 A portion of the apical cytoplasm of a cell from Kolliker's organ showing numerous thick microvilli (*mv*) and a small kinocilium (*k*) which shows a basal body (*b*) and a centriole (*c*) The core of the microvilli contains a fine fibrillar structure that projects into the apical

×23 460



crowded at the basal end (Figs 2 3) The cytoplasm contained a large number of vesicles elongated mitochondria and various organelles (Figs 2 3)

The tectorial membrane covered Kolliker s organ completely (Fig 2) In some regions the fibers of the tectorial membrane appeared to be attached rather loosely to the microvilli of the apical plasmalemma by a network of fine filaments (Figs 4 5) This loose filamentous network filled the space between the cells surface and the tectorial membrane During the

Fig 6 Junctional complex between two adjacent cells of Kolliker's organ *ZO* Zonula occludens *ZA* Zonula adherens ×36470

Fig 7 Another junctional complex showing a zonula occludens (*ZO*) a zonula adherens (*ZA*) and a small desmosome (*D*) ×36708

Fig 8 This junctional complex exhibited a zonula occludens (*ZO*) and a well developed desmosome (*D*) ×67037

r stages of the progressive disappearance columnar cells the contact of the filamen is network with these cells seemed to be lost in most areas but not in all (Figs 11 12)

Observations on the cellular level were as follows Cellular degeneration was suggested by the appearance of autophagic vacuoles containing granules of various sizes amorphous substances and a variety of cell organelles (Figs 9 10 11 13) In some vacuoles such organelles as endoplasmic reticulum

Fig 9 An autophagic vacuole at high magnification limited by a double unit membrane The vacuole contains mitochondria smooth and rough endoplasmic reticulum and ribosomes At the arrows the membranes are not apparent ×12770



Fig 10 Group of cells of Kölliker s organ containing nu ... stages of de ve ... partially su ... (2) contains endoplasmic reticulum and ribosomes Vacuoles (3 to 7) consist of numerous vesicular profiles filled with both amorphous and electron-dense materials The membrane sur... large ... regular ... with gran... The vac... branes ...

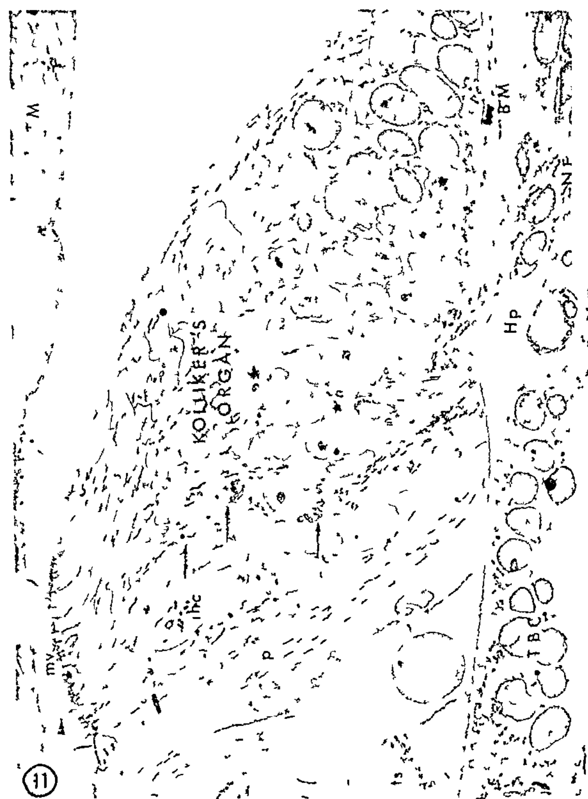


Fig 11 Low po er elect n m graph f K l ker s organ (ap cal t rn) of nes l k en h w ng a marked reduct on in the numb f e mp red w th F g 2) The ap cal port on f rf e f the re ma n ng cells sho vs numerous m crov ll (*m*) The tec torial membrane s attached to the m cro ll of s me of the rema n ng cells (*arrow l ea l*) In the cyt plasm some a tophagic vacuoles can be seen at the arrows ×1489

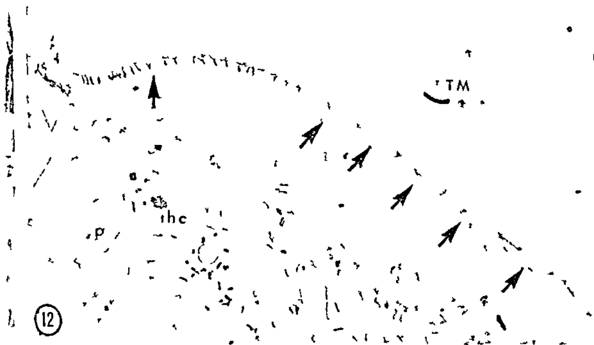


Fig 12 Apical portion of Kolliker's organ of one-day old kitten showing numerous areas of contact (arrows) between the tectorial membrane and the free surface of Kolliker's cells ×1 567

ribosomes and mitochondria could still be recognized (Fig 9) Other vacuoles were made up of numerous vesicles filled with amorphous material or electron dense substances Still other autophagic vacuoles were composed of irregular membranous structures and large vesicles containing fine granular material (Fig 10) Most of the autophagic vacuoles described were surrounded by a double unit membrane but in a few instances the vacuoles had a single unit membrane the latter usually contained a rather dense membranous material Such autophagic vacuoles were seen in all stages of change occurring within Kolliker's organ but were more abundant in the early stages (Figs 11 13) The replacement and reduction of numbers of cells in the organ were followed by the formation of progressively larger intercellular spaces (Figs 13 14) until the whole Kolliker's organ was replaced by inner sulcus epithelium (Fig 15) The latter seemed to originate from the cells of the former but as in Hensen's study we were unable to follow the replacement process Some preparations (as in Figs 13 and 14) suggested that some cells of Kolliker's organ changed from high cylindrical shape to low cuboidal but again that such a process actually occurs remains in question

DISCUSSION

Because we were unable to determine the exact nature of the apparent process of transformation occurring in Kolliker's organ as a whole specifically whether such a process involved a degeneration degradation regression or actual disappearance of this organ such a change in the organ will be simply referred to in this discussion as a transformation Hopefully further research in this area will clarify this process more precisely

It is possible this transformation may represent a case of localized morphogenetic degeneration as described by Glucksmann (1951) involving ontogenetic cell death which participates in shaping the form of an organ in this case the inner sulcus and the tectorial

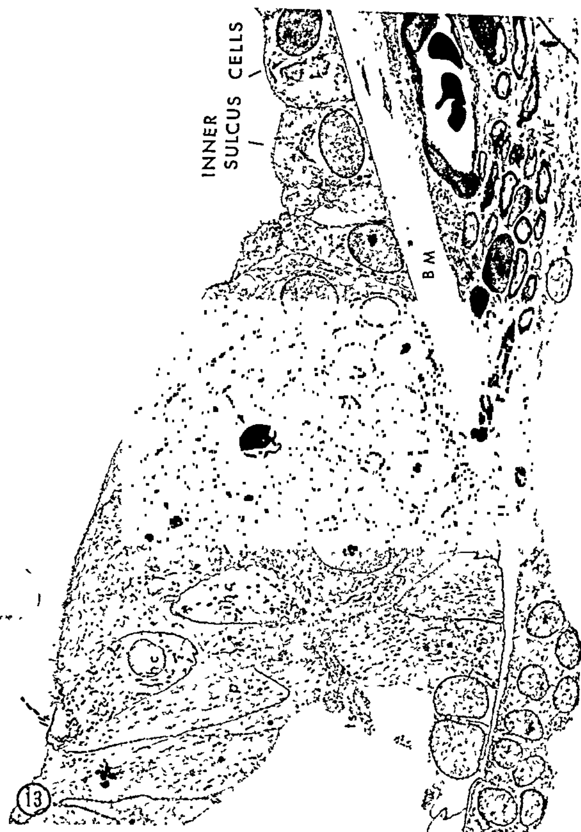


Fig 13 Low power electron m r graph of Kolliker's organ (apical turn) of an 11-day-o s en showing further reduction in number of cells and i ition of large inter cellular spaces between them Note the presence of nu merous autophagic vacuoles (*arrows*) ×1757

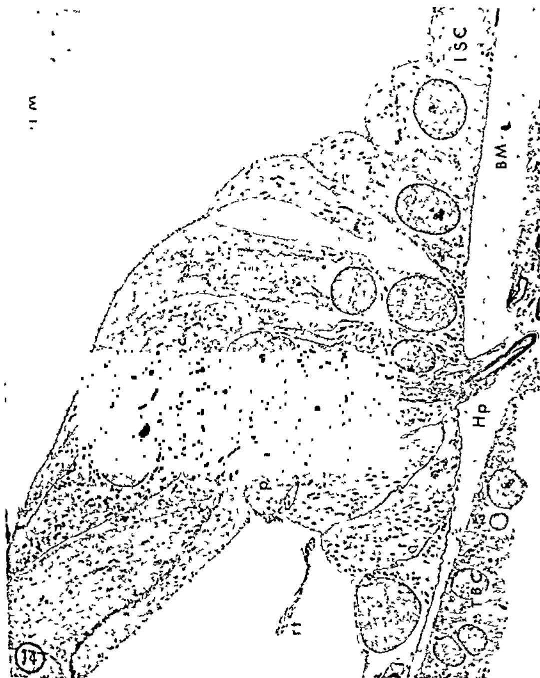
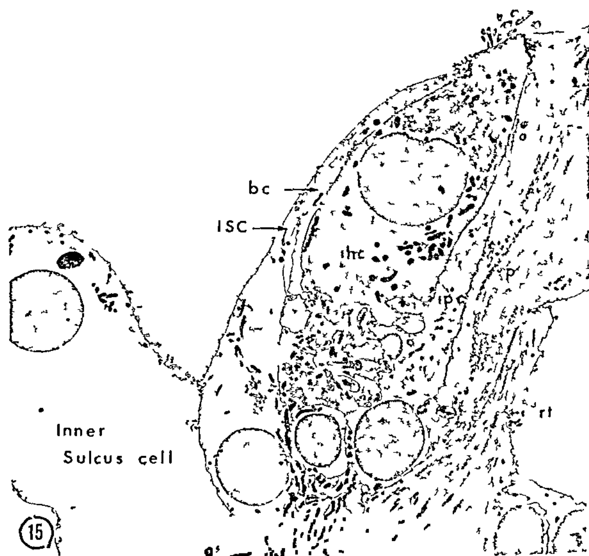


Fig. 14. Low-power electron micrograph of Kölliker's organ (apical turn) of a 20-day-old kitten. It shows the reduction of Kölliker's organ to 3–4 rows of cells with large intercellular spaces between them. ×1990

membrane. However, such comparison regarding the process of transformation of the organ as a whole must await more specific evidence before this term can be applied to our observations.

In contrast, on a cellular level, our study provides ample evidence for describing the nature of the transformation occurring in Kölliker's organ, specifically as that involving a process of cellular autophagocytosis (a term



5 Low po er electron m crograph of the inner hair d support ng elements of the adult cat The inner ell (*hc*) is surrounded by the inner phalangeal cell (*pc*) and the border cell (*bc*) The latter is in contact w h the slender process of the first inner sulcus cell (ISC) ×2 777

introduced by Arstila & Trump 1968) result ing in widespread cell death and total disap pearance of Kolliker s organ

This process although termed differently by various investigators as cellular autophagy (DeDuve & Wattiaux 1966) and focal cyto plasmic degradation (Hruban 1963) will simply be referred to in th paper as cellular autophagocytosis

Cellular autophagocytosi has been recog nized as a process whereby l ing cells achieve the degradation of their wn cytoplasm (DeDuve & Wattiaux 1966) This process has been observed under normal condit ons (DeDuve & Wattiaux 1966) The rate of such normal autophagocytosis however is often increased in certain processes such as re modeling during metamorphosis (Bonneville 1963) development (Moe & Behnke 1962) differentiation (Behnke 1963) aging (Brandes 1966) and atrophy (Brandes 1963) However cellular autophagocytosis have also been observed in pathological conditions (Arstila & Trump 1968)

The actual process of cellular autophago cytosis consists of five morphological stages

(1) sequestration of a portion of cytoplasm by two isolating membranes (2) formation of a single limiting membrane (3) morphological alterations of the sequestered cytoplasmic components (4) transformation of autophagic vacuoles into lysosomes and (5) transformation of lysosomes into residual bodies (for review see DeDuve & Wattiaux 1966 Hruban & Rechcigl 1969) All of these morphological stages were seen to occur in the transformation of Kolliker's organ observed in our specimens

The first three stages of this process are morphologically distinct and referred to as autophagic vacuoles defined as membrane bound structures containing cytoplasmic organelles or subcellular systems in various stages of digestion (DeDuve 1963) The origin of the membrane enclosing autophagic vacuoles has been the subject of considerable controversy A number of researchers in this area however have suggested that the sequestering membranes are formed by the endoplasmic reticulum (Hruban et al 1962 Novikoff & Shin 1964 Ericsson et al 1965 Beaulaton 1967 Arstila & Trump 1968) As mentioned above after this first stage the two limiting unit membranes are transformed into a single unit membrane during the second stage Arstila & Trump (1968) have suggested that the single unit membrane in the autophagic vacuoles is derived from the Golgi complex Regarding the third stage of these vacuoles the morphological alterations of the sequestered organelles seem to suggest degradation and digestion by hydrolitic enzymes (Hruban et al 1962 1963 Swift & Hruban 1964 Biava 1965) During the fourth stage such enzymes appear to be responsible for the advanced breakdown of the sequestered organelles At this point the autophagic vacuoles seem to be lysosomes Finally during the fifth stage these lysosomes are transformed into residual bodies after complete digestion of the sequestered materials has occurred Thus as observed in many studies the process of autophagocytosis is characterized by a progressive volume reduction of the vacuoles and dense bodies (Hruban & Rechcigl 1969)

As described previously in this paper our electron microscopy observations indicate that Kolliker's organ and the tectorial membrane are connected through a network of fine filaments in the embryo As the transformation of Kolliker's organ progresses the contact of the fine filamentous network with the microvilli at the level of the replaced columnar cells appears to be lost leaving only a few points of contact in the newborn kitten Kikuchi & Hilding (1965) describe a similar filamentous network between the tectorial membrane and the supporting cells of the outer segment of the organ of Corti of the developing mouse cochlea According to their study the outer or marginal attachments are found in the newborn mouse but apparently disappear as the maturation of the organ of Corti progresses However the relationship between the tectorial membrane and Kolliker's organ is not discussed by these authors

The question of whether the tectorial membrane is actually attached to the area of inner hair cells and their supporting cells has been discussed by a number of investigators Kimura (1966) studied human and animal specimens including the newborn mouse and found no evidence of attachment of the tectorial membrane to the area medial to and including the inner hair cells Lindeman et al (1971) studied the area medial to the inner hair cells in the newborn kitten also *finding no* remnants to indicate any attachment of the tectorial membrane to this area On the other hand Lim & Lane (1969) and Lim (1972) have described in guinea pigs cats and monkeys regularly arranged trabeculae *in the* Hensen's strip that are assumed to function as anchoring devices between the tectorial membrane and the supporting cells surrounding the inner hair cells

According to the work of Iurato (1967) *and* Ross (1974) with *rats* and in that of Hoshino (1976) with cats such actual physical contact between the inner hair cell hairs and the tec

torial membrane has been suggested However, in our own study, while attachment between the tectorial membrane and the supporting cells around the inner hair cells seems indicated, the involvement of the inner hair cell hairs themselves in this contact remains in question

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ZUSAMMENFASSUNG

Die Struktur und postnatale Umformung de Kollikerschen Organes in der Katze wurden sowohl mit dem Licht als auch mit dem elektronischen Mikroskop untersucht Das Kollikersche Organ welches sich wahrend der embryonischen Entwicklung des Katzchens in der inneren Furche des Ductus Cochlearis befindet scheint wahrend der ersten 30 Tage nach der Geburt eine grossere Umformung mitzumachen Vom Standpunkt des Organes als Ganzes werden die Möglichkeiten dieser Umformung hier kurz beschrieben Vom zellularen Standpunkt aus gesehen macht das Organ einen Prozess zellularer Autophagocystose mit Das Kollikersche Organ besteht aus vielfachen eng gewobenen und langen saulenförmigen Zellen welche die innere Furche ausfüllen Autophagische Vakuolen welche Zellorganellen enthalten wurden in diesen Zellen wahrend des gesamten Umformungsprozesses beoabachtet Die zelluläre Autophagocystose reduzierte die gegenwartigen Zellen jedes Abschnittes von ca 50 im neugeborenen zu ca 12 im 30 Tage alten Katzchen Die Umformung des Kollikerschen Organes vollzog sich offensichtlich vom Grund zur Spitze und vom Limbus zur inneren Haarzelle Die Beziehung des Tektorialmembranes zum Kollikerschen Organ und die des Tektorialmembranes zur Umgebung der inneren n werden ebenfalls beschrieben

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List of abbreviations BM, Basilar membrane bc, Border cell Hp, Habenula perforata ihc, Inner hair cell ipc Inner phalangeal cell ISC, Inner sulcus cell NF, Nerve fibers in osseous spiral lamina n, Nucleus ohc, Outer hair cell P, Pilar cell rt, Radial tunnel bundle TM, Tectorial membrane ts, Tunnel spiral bundle TBC, Tympanic border cells

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VARIABLES AFFECTING THE DRILL-GENERATED NOISE LEVELS IN EAR SURGERY

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Abstract *The manner in which the variables rotation* speed type of burr burr size and site of drilling influence bone conducted drill generated noise levels in ear surgery has been investigated From the results obtained the following conclusions can be drawn

1 The noise levels were primarily influenced by the size of the burr The 6 mm cutting burrs gave a noise level of 88–108 dB the use of a 4 mm one resulted in a reduction of 1–6 dB and the use of a 2 mm one 5–16 dB The mean noise levels of the diamond burrs were 5–11 dB lower than the mean noise levels of the cutting ones

2 Variations in rotation speed had only a slight influence on the noise levels produced (0–5 dB)

3 Three different types of cutting burr were tested All gave noise levels of about the same order of magnitude

4 The noise levels around the cochlea were only tly influenced by the localization of the drilling the ear (< 1 8 dB)

Drill induced noise levels in ear surgery cannot be uced to any great extent Possible noise traumas to e inner ear can only be avoided by minimizing the duration of drilling and thus *the duration of harmful* noise exposure to the cochlea

Experiments concerning the noise levels produced by drilling in temporal bones have earlier been described by Paulsen & Vietor (1975) on isolated temporal bones and by the present authors (Kylen & Arlinger 1976) on intact skulls of human cadavers The results of these two investigations differ to a marked degree Drilling on intact skulls produced much higher estimated noise levels than that on temporal bones—about 100 dB compared with 60 dB

In our previous study the experiments on intact skulls were performed with a drill rotating at about 20 000 rpm Two kinds of burrs with a diameter of 6 mm were employed, a cutting one with 12 cutting edges and a diamond one with a stone size of 120 μm

The aim of the present investigation was to study to what degree the noise levels produced vary with rotation speed, type of burr size of burr and site of drilling

METHODS

Preliminary drillings were made on isolated temporal bones The main experiments were performed on eleven ears of six human cadavers with intact skulls and ears The recording equipment used in this study is described in an earlier paper (Kylén & Arlinger, 1976)

A miniature accelerometer (Bruel & Kjaer 8303, weight 3 5 g) was screwed on to a brass rod (length 35 mm, diam 1 8 mm weight 1 g) which was firmly fixed to the promontory by means of cranial cement The signal from the accelerometer was amplified (Bruel & Kjaer 2603) and fed to a tape recorder (Revox A77, 19 cm/sec) The tape recordings were analysed off line using an octave band filter (Bruel & Kjaer 1612) con

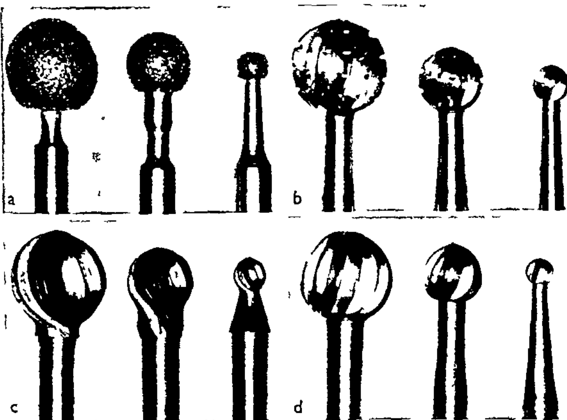


Fig 1 Four types of burr were used in the experiments Each type is represented by three specimens with a diameter of 6 4 and 2 mm respectively (*a*) Type 1 Diamond burrs Stone size 120 μm (*b*) Type 2 Cutting burrs with notched edges 6 mm 12 cutting edges (c e) 4 mm 12 c e 2 mm 8 c e (*c*) Type 3 Cutting burrs 6 mm 12 c e 4 mm 12 c e 2 mm 7 c e (*d*) Type 4 Cutting burrs 6 mm 14 c e 4 mm 12 c e 2 mm 8 c e

nected to the amplifier (Bruel & Kjaer 2603) and level recorder (Bruel & Kjaer 2305)

Three types of air rotors have been used The Cyclone 160, giving about 16000 rpm, the Cyclone 220, giving ca 20000 rpm and the Cyclone 300, giving ca 25000 rpm Four different types of burr were selected for the experiments, each type containing three specimens with a diameter of 6 mm 4 mm and 2 mm respectively (Fig 1*a*–*d*) The drilling was done in the part of cortical bone covering the cell system of the ear This area was divided into three smaller portions (Fig 2) In every ear each burr was used three times per area once with 16000 once with 20000, and once with 25000 rpm i e 108 short bursts of drilling per ear The static force applied to the drill was well over 4 N (0 4 kP)

The results of our previous study were given in dB equivalent air-borne octave levels and concerned a 6 mm cutting burr at a rotation speed of about 20000 rpm The burr has been classified in this study as type 3, 6 mm (Fig 1*c*) In the present study we have calculated the noise levels from the vibration signals of this burr at 20000 rpm in a manner described in our former study (Kylén & Arlinger, 1976, Fig 3)

In analysing the results of the present study (Fig 4*a*–*l*) the noise levels produced by the 6 mm, type 3 burr at a rotation speed of 20000 rpm are used as reference values, i e the noise levels obtained from the other



Fig 2 The cortical bone covering the cell system divided into three areas 12 burrs have been used 3 times per area—108 drillings per ear

drilling variables are compared with these reference values This procedure allows an easily comprehensible display of the influence of the different variables By means of the data displayed in Fig 4*a–l* and the data in Fig 3, the equivalent air borne octave levels for a specific set of drilling parameters can be obtained

An analysis of variance was made to investigate the influence of the four independent variables—the rotation speed the type and the size of the burr and the site of drilling These calculations were made with the `of a computer

RESULTS

e noise performance of the diamond burr (Fig 1*a*) differs to a great extent from that of the cutting burrs (Fig 1*b–d*) The mean noise levels of the diamond burrs are 5–11 dB lower than those of the cutting ones (Table I)

The variables size and rotation speed influence the noise of the diamond burrs and the cutting burrs in different ways We will therefore describe the results from those two groups separately

Diamond burrs

In the 0 5 kHz and 1 kHz octave rotation speed and burr diameter influence significantly the noise levels produced However these factors explain only a small part of the variance in the experiment—10% for the 0 5 *kHz* band and *36%* for the *1 kHz* octave band In the higher frequency bands (2 4 and 8 kHz) these variables account for the major part, 65–77%, of the variance and we will therefore consider only these higher octave bands when reporting on the results concerning the diamond burr The effects of these two variables on the noise performance are significant ($p<1\%$) There are no significant interaction effects

The rotation speed has only a small influence on the noise levels, the difference between 25000 and 20000 rpm being negligible (<0 5 dB) The use of 16000 rpm gives a reduction of 2–4 dB compared with the other rotation speeds tested The results are significant ($p<1\%$)

The most important variable is the diameter of the burr, accounting for 96–97% of the explained variance (Fig 4*a–c*) The reduction in noise levels when using a 4 mm or a 2 mm burr compared with a 6 mm one is shown in Table II

The site of drilling (Fig 2) has an insignificant influence on the mean noise levels produced ($p<1\%$)

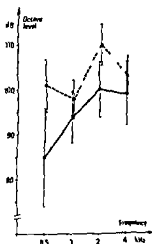


Fig 3 Equivalent air borne sound levels (octave levels) generated by a type 3 *6 mm burr* at a rotation speed of 20000 rpm Mean values ±*1* S D Former study (—) Present study ( ) For comments see text

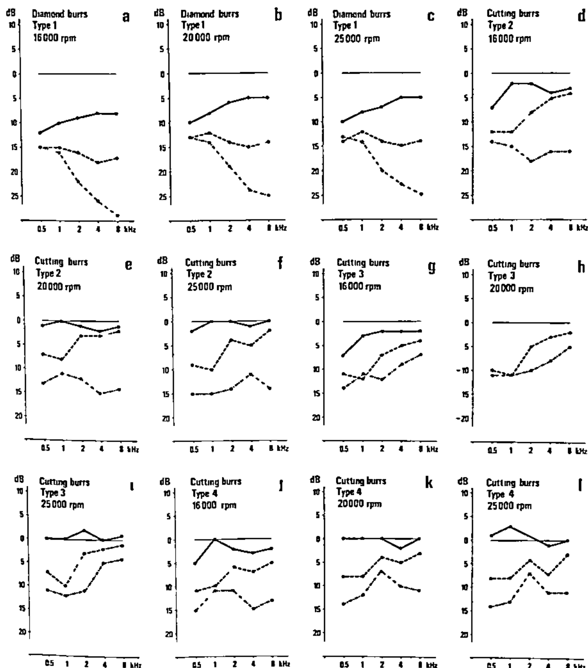


Fig 4 (*a–l*) Difference in equivalent air borne octave levels between the type 3 6 mm burr at a rotation speed of 20000 rpm and the noise levels obtained from the other drilling variables (rotation speed type of burr and size of burr) 6 mm(—) 4 mm ( ) 2 mm ( )

Cutting burrs

The results of our statistical model implied in the analysis of variance show that the noise levels of the cutting burrs depend on the variables speed, type and size, in a very complicated way This is particularly due to the influence of bifactorial interactions (diameter–type and diameter–speed) These

Table I *Difference in dB between the mean noise levels produced by the cutting burrs and the diamond burrs*

Octave band (kHz)	Reduction (dB)
0 5	5
1	5
2	8
4	10
8	11

significant, but have only a slight effect on the noise levels produced However, they force us to consider the combined influence of the parameters speed, type and size on the noise levels when reporting on the results of these experiments The results are displayed in Fig 4 and the influence of the variables is significant ($p<1\%$) The model explains 42–62% of the variance in the experiment, which is less than the explained variance of the diamond burrs

We find that the rotation speed has a very slight influence on the noise levels of the cutting burrs In the most hazardous octave bands (2, 4, and 8 kHz) the difference is in the range 0–5 dB, the 16000 rpm giving the owest and the 25000 rpm giving the highest ls ($p<1\%$, Fig 4) The results from our iminary studies on isolated temporal és indicated that the use of still higher rotation speeds (32000 and 50000 rpm) gave noise levels of the same order of magnitude as those produced in the 16000–25000 rpm range

Table II *Difference in dB between the mean noise levels of the 6 mm diamond burr and the 4 mm and 2 mm ones*

Octave band (kHz)	Reduction (dB)	
	4 mm	2 mm
2	6	13
4	10	18
8	9	20

Table III *Difference in dB between the 6 mm cutting burrs and the 4 mm and 2 mm ones Mean values, all types included*

Octave band (kHz)	Reduction (dB)	
	4 mm	2 mm
0 5	7	11
1	10	12
2	4	11
4	3	9
8	2	10

The noise levels vary only slightly with the *different types of cutting burrs, 6 and 4 mm* diameter (0–4 dB) However, this is not the case with the 2 mm cutting burrs For these the spread in noise levels obtained in the higher frequencies (4 and 8 kHz) is considerable, type 3 giving noise levels that are 5–10 dB higher than type 2 and type 4 (Fig 4*d–l*)

As in the case of diamond burrs the size of the burr has the greatest effect on the noise levels produced (Fig 4*d–l*) The 6 mm cutting burrs give about 100 dB sound level *in the 2–4 kHz bands, in the lower frequency* bands somewhat less The use of a 4 mm cutting burr gives a reduction of 1–6 dB in the 2–8 kHz bands and the use of a 2 mm cutting burr 5–16 dB in the same octave bands The mean reduction in noise level with smaller burr diameters is shown in Table III

The influence of the drilling site on the noise levels produced is not significant in some octave bands It is statistically significant in other bands, but of very little importance (<1 8 dB, $p<1\%$)

DISCUSSION

By employing intact skulls of human cadavers we have imitated the conditions of real ear surgery as far as possible At first we conducted the studies on isolated temporal bones However, we found that the variations in the results, comparing one temporal

bone with another, were so great, that they concealed the influence of the variables that we were studying

We have excluded rotation speeds below 16000 and above 25000 rpm from this study We feel that the reduced cutting speed of a drill performing less than 16000 rpm renders it unfit for ear surgery Moreover, high-speed drills (>25000 rpm) in ear surgery have a tendency to 'kick-off', i e turn themselves out of the hands of the surgeon and land in unfavourable places, and 'throw-off', i e pieces of the burr s cutting edges shear off and spin away The higher rotation speeds are therefore not recommended in ear surgery These views are in agreement with those of Hallen & Tjellstrom (1975)

The three types of cutting burrs were selected on grounds of their differing characteristics, such as the number of cutting edges and the construction of the edge itself (Fig 1*b–d*) We originally planned to include an 8 mm burr in the studies but this diameter was not available in all the types and was therefore excluded

The 250 Hz octave band has also been omitted from this investigation because the smaller burrs seldom show readings above background level at this frequency However, the results from the 8 kHz octave bands have been added for comparison At a static force of about 4 N (0 4 kP) on the drill, approximately maximal noise levels are noted (Kylen & Arlinger 1976) Great care has been taken in the present study to exceed this static force every time the drill was used We find that the noise levels of the type 3, 6 mm burr at 20000 rpm, i e the results linking this study with our former one, are slightly higher in the present study than in the earlier one (Fig 3) This is due to the fact that in this study very short bursts of drilling were used and therefore the readings are maximal values In the previous study only two types of burr were used This permitted drilling for much longer periods of time in each ear When evaluating the recorded noise levels, average values were selected instead of maximal values Reference values in the diagrams (Fig 4) are based on the present results but all the equivalent air-borne noise levels mentioned in the text are based on the results of our earlier study This permits the use of average levels instead of maximum ones

The variables rotation speed and burr size account for 65–77% of the variance in the noise levels produced by the diamond burrs The noise levels from the cutting burrs are influenced by the variables of speed, type of burr and size of burr and 42–62% of the variance is attributable to these variables The bone structure varies from ear to ear and this is probably the major factor behind the unexplained part of the variance It probably also accounts for the fact that a greater part of explained variance was found in the experiments with the diamond burrs These burrs have a smoother surface than the cutting ones, which, upon meeting bone of different structures, jump and bounce considerably more than diamond burrs do In this way the cutting burrs are likely to produce vibrations not accounted for by the variables in our model However, we have been unable to standardize this bone structure variable, which has therefore been excluded from the investigation

Of all the variables, size of burr has the most pronounced effect on the noise levels The effect of the different variables is clearly shown in Fig 4*a–l* In comparison with the size of the burr, all the other variables have little effect on the noise levels produced Thus, the rotation speed of the drill has a very slight influence (in the 2, 4, and 8 kHz octave bands, 0–5 dB) The cutting burrs with a diameter of 6 mm show very small variations and all produce equivalent air borne octave levels of approximately 100 dB (88–108 dB) in the 2–4 kHz bands The 4 mm ones all give a reduction of 1–6 dB, i e often causing noise levels in the order of 100 dB The 2 mm cutting burrs give noise levels of

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LONG-TERM RESULTS AFTER STAPEDECTOMY

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Abstract 296 cases of stapedectomy accomplished by vein grafting plus polyethylene tubing with a primary (one year) result of a >10 dB improvement in Air Conduction (AC) threshold in speech frequencies have been tested with pure tone audiometry 1–12 years postoperatively The AC threshold of the operated ear increased by about 1 dB per year for both low and high frequencies In the high frequency range this increment can be ascribed to normal aging in the low range the reason is probably progressive conductive loss There is no evidence to support the assumption that the operation impairs the sensorineural function of the operated ear The AC threshold of the unoperated ear also increased—by more than that of the operated ear for both low and high frequencies In the low range this was ascribed to progressive stapes fixation in the high range to stapes fixation plus normal aging The progression for the non-operated ear is relatively slow about 2 dB per year

fore than twenty years have passed since hea in 1955 performed his first fenestration of the oval window with reconstruction of the sound-conducting mechanism of the middle ear Since then hundreds of thousands of operations of this kind have been carried out and the literature is replete with reports on the results Many authors however point out the difficulties encountered in making a satisfying follow up investigation on these patients Kos (1969) for example reports vein plug stapedectomy results in a statistical study with almost 70% drop-outs for a multitude of reasons' Shambaugh said (1969) in a long term study on different techniques The fenestrated patient must return for the rest of his life to the surgeon or to another otologist for care of his operated cavity and this affords an ideal opportunity to collect long term results on an unselected group of patients, for the good and the bad results patients have the same need for care of the cavity On the contrary, the successfully operated patient tends not to return unless he runs into trouble with vertigo or loss of hearing Any long term study of stapes operations tends to be weighed toward poor results"

Most of the numerous reports on the results of stapedectomy are therefore characterized by short observation time The most important problem seems to be "the air–bone gap problem", which naturally in itself is a very valuable parameter for comparing different techniques, but it does not say everything about the patient's hearing

Paparella (1974), editor of the Year Book of ENT, points out, in an editorial comment on a paper by Sooy et al (1973), that "long term studies like this one are uncommon in the literature It would be desirable if other authors would present their long term results This information becomes useful in advising patients preoperatively as to the general possibility of a successful long term result

On account of this comment the following report is given, based on material judged as suitable for this purpose

MATERIAL

From 1961 to 1975 over 400 ears with the diagnosis of clinical otosclerosis have been

Table I *Age distribution*

Age at op	0–39	40–49	50–59	≥60
Number	59	64	91	82
Percentage	19	23	30	28

operated upon using the same technique, viz stapedectomy with vein graft and polyethylene tubing The only difference from the original technique described by Shea (1956) is that the tube is cut off straight at both ends and not with a sharp tip at the medial end, so as to avoid the risk of perforation of the vein In the following report only the first 353 ears are considered, because of the need to have more than one year's follow-up 63% of the patients were female and 37% male, which is in accordance with the usual distribution in most reports The age distribution is shown in Table I More than half of the patients were over 50 years old at operation

PRIMARY RESULTS

For the 353 ears operated in 1961–73, the primary (one year) results are shown in Table II, which shows that after one year, 84% displayed an improvement of 10 dB or more for AC PTA, i e Pure Tone Average of Air Conduction on operated ear measured at 500, 1 000 and 2 000 Hz

The group "unimproved because of sensorineural component" is a group of failures which strangely enough is never found mentioned in reports on the results It has earlier been pointed out (Nilsson, 1970) how difficult it is to establish a reliable bone conduction threshold in cases of maximal conductive deafness in one ear and sensorineural impairment in the other In such cases the risk must always be present that an operation is made on an ear where the sensorineural component is more pronounced than is shown by the bone conduction measurements In our material this group is as large as 10%, which requires further explanation

All these cases had bilateral, pronounced hearing loss and the worse ear was always operated upon Weber's test was mostly uncertain In most cases the speech discrimination score was relatively low Nevertheless, these patients were operated upon either because they insisted on it, or because the hearing with the relevant ear was so bad that the patient had everything to gain and nothing to lose However, if one is critical, these cases must be regarded as preoperative misjudgments As can be seen in Table II the number of these cases has decreased over the years, step by step as the diagnostic criteria have been improved In the period 1961–64 they amounted to 13%, in 1970–73 to 5% The number of "technical failures", where the operation could not be completed, mostly because of "impossible" footplate, has been almost constant over the years

LONG TERM RESULTS

It has been pointed out earlier that reports on long term results are rare

Kos (1969) reported 117 vein plug stapedectomies, with an average decrement of 1 3 dB from one to six years postoperatively for the mean of the speech frequencies (500, 1 000, 2 000 Hz) At 4 000 Hz a decrement of 13 dB occurred during the same period

Sooy et al (1973) reported 8-year results following wire vein graft stapedectomy No significant decrements in pure tone thresholds were shown over the 8 years for the speech frequencies (500, 1 000, 2 000 Hz) Decrements

Table II *Primary (one-year) results*

	No	Per centage
Improvement 10 dB or more	296	84
Unimproved		
sensorineural component	36	10
"technical failure	10	3
Deterioration 10 dB or more	3	0 8
Drop-out (not checked after 1 year)	8	2 2
Total	353	100

Table III *Percentage ' Sensorineural component ' and "Technical failure in relation to year of operation*

	61	62	63	64	65	66	67	68	69	70	71	72	73
Percentage Sensorineural component	20	9	11 13	15	8	9	12 10	13	11	6	0 5	6	5
Percentage Technical failure			2				2				4		

of approximately 0 5 dB and 1 dB per year occurred for 4000 Hz and 8000 Hz respectively These results indicated, according to the authors, a good stability of hearing over the 8 years

The pure tone audiometry results during the subsequent years were studied on the above-mentioned 296 ears where an improvement of 10 dB or more in AC PTA was shown after one year The AC threshold for 250, 500, 1000, 2000, 3000 and 4000 Hz on operated and unoperated ears and 'best bone conduction (BC) without masking" for the same frequencies have been analysed statistically The whole material consists of about 30000 measurements The motivation for using "best BC without masking is that masked BC measurements from different testing occasions are difficult to compare Best BC without masking" naturally does not always represent the ear actual for operation

As a source of error in an investigation like this however this can only lead to an under statement of the result of the operation, never to an overstatement If on the other hand pre and postoperative BC measurements are made with masking of the opposite ear, the results are uncertain The effect can for example be a phenomenon often described as 'overclosure of the air–bone gap This means that the postoperative AC threshold is lower than the preoperative BC threshold

In Fig 1 it can be seen that the AC PTA in operated ears shows an average of 29 dB gain for all cases after one year During the following 12 years the hearing slowly decreases with about 10 dB It is also shown in this figure that the group with a high preoperative threshold gains more than that with a lower preoperative threshold, but naturally they both end up at about the same level

The change in the air–bone gap is shown in Fig 2 The gap increases slightly, but not as much as the AC loss which is explained by increased BC threshold (see Fig 5)

Fig 3 shows what happens to the AC of the operated ear at different frequencies The biggest gain is naturally made in the low frequencies but even 4000 Hz gets a considerable improvement—more than 10 dB, on average One interesting fact is that the decrease during the following years affects all frequencies to the same extent

A question seldom discussed is what hap-

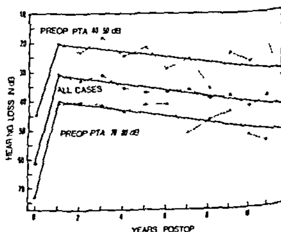


Fig 1 Mean values for AC PTA (pure tone average for 500 1000 2000 Hz) on operated ear before (0) and 1-12 years after operation for all cases for a group with good preop PTA (40–50 dB) and another group with bad preop PTA (70–80 dB) Mean values for all measurements — the linear regression for these values

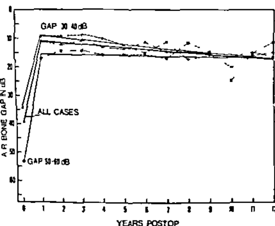


Fig 2 Mean values for air-bone gap on operated ear PTA for all cases for a group with preop gaps of 30–40 dB and another group with preop gaps of 50–60 dB Mean values for all measurements, — the linear regression of these values

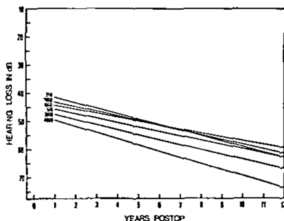


Fig 4 Mean values for AC at different frequencies on unoperated ears 1–12 years after operation Only the linear regression of the mean values is drawn, in order to make the diagram more clear

pens to the contralateral ear This is depicted in Fig 4 Here it can be seen that the AC threshold increases for all frequencies and more than in the operated ear (15–20 dB in 10 years) because of the progressing otosclerotic process

Fig 5 shows how the BC ("best BC without masking") threshold increases for all frequencies and significantly more for the higher ones The results of this follow-up study, graphically demonstrated in Figs 1–5, are summarized in Table IV

Table IV shows the average increase of pure tone thresholds for various frequencies in dB per 10 years for "best BC without masking", and for AC of operated and unoperated ear. Another method to make the results clearer is used in Figs 6, 7 and 8 Here the data for threshold measurements are presented as "average audiograms"

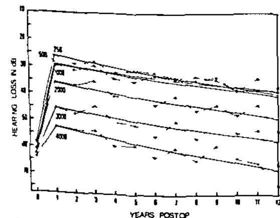


Fig 3 Mean values for AC at different frequencies on operated ear before (0) and 1–12 years after operation Mean values for all measurements — the linear regression of these values

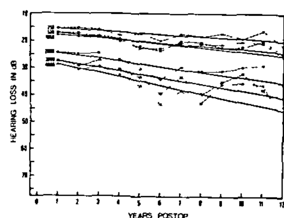


Fig 5 Mean values for BC at different frequencies 1–12 years after operation Mean values for all measurements — the linear regression for these values

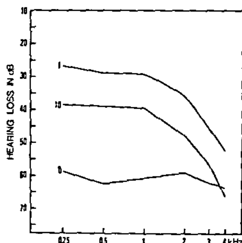


Fig 6 Average pure tone *AC* audiogram of the operated ear for all cases before (0) and 1 and 10 years after operation

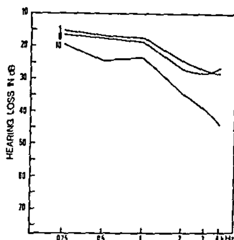


Fig 8 Average pure tone BC audiogram for all cases before (0) and 1 and 10 years after operation

DISCUSSION

The present investigation confirms earlier published results by Kos (1969) and Sooy et al (1973) showing a slowly progressing increment of the AC threshold of the operated ear by about 1 dB per year for low as well as high frequencies The BC threshold does not increase to the same extent in the low frequency range, which indicates that the increase in the AC threshold is of conductive origin The cause may be increasing stiffness of the ossicular chain, or the new oval window membrane, or it can be further progression of the otosclerotic disease The unoperated ear also shows an increase in the AC threshold amounting to about 2 dB per year This is important information for the patient when discussing the prognosis of the hearing loss The increase in the high tone loss of both the operated and the unoperated ear (1–2 dB per year) is no greater than can be expected with regard to the age of the patient

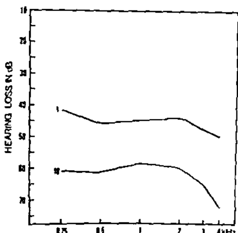


Fig 7 Average pure tone AC audiogram of the unoperated ear for all cases 1 and 10 years after operation

ZUSAMMENFASSUNG

292 Stapedektomien mit Venengraft und Polyethylenröhrchen die eine primäre (1 Jahr) Verbesserung der Luftleitung von mehr als 10 dB in den Sprachfrequenzen erreichten sind postoperativ ein bis 12 Jahre mit Tonaudiometrie kontrolliert worden Die Luftleitungsschwelle des operierten Ohres stieg mit ungefähr 1 dB pro Jahr sowohl im Bereich der niederen als auch der hohen Frequenzen Im Bereich der hohen Frequenzen kann diese Einschränkung dem normalen Altern zugeschrieben werden in den niederen Frequenzbereichen ist wahrscheinlich die fortschreitende Verschlechterung der Schalleitung

Table IV *Average increase of pure tone threshold for different frequencies in dB per 10 year*

Frequency	250	500	1 000	2 000	3 000	4 000
BC	4	7	6	10	12	16
AC op ear	12	9	11	12	11	14
AC unop ear	20	16	14	16	17	22

die Ursache Es gibt keinen Anhaltspunkt für die Annahme daß die Operation die sensorineurale Funktion des Ohres schadigt Die Luftleitungsschwelle des nicht operierten Ohres stieg namlich auch, und zwar mehr als die des operierten Ohres sowohl in den niederen als auch in den hohen Frequenzen Dieser Umstand kann im niederen Frequenzbereich einer fortschreitenden Stapesfixation und im hohen Frequenzbereich der Stapesfixation einschließlich dem zunehmenden Alter zugeschrieben werden Der Schwellenanstieg im nichtoperierten Ohr schreitet mit ungefahr 2 dB pro Jahr vor

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MEASUREMENTS OF THE AIR CONDITIONING CAPACITY OF THE NOSE DURING NORMAL AND PATHOLOGICAL CONDITIONS AND PHARMACOLOGICAL INFLUENCE

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Abstract A simple method is introduced for measuring the air conditioning capacity of the nose A flow of 8 l/min dry air is introduced by a catheter into the nasopharynx while 5 l/min is sucked out from the investigated nasal cavity through a psychrometer The additional 3 l/min passes down into the pharynx thus reducing the inter mingling with expiratory air By using CO_2 as a tracer this error was found to be maximally 15% and often about 1% The three different enthalpy factors increase in en thalpy of dry air vaporization and increase in enthalpy of water vapour were calculated separately and the vaporization was found to be the dominant factor The calculated total supply of humidity showed that the method presented causes at least a slight stress on the humidifying capacity Pharmacological studies have shown that subcutaneously injected atropine decreased the total enthalpy and that of water vapour while nasal administration of oximetazoline also decreased the total enthalpy Nasal administration of homatropine or pilocarpine had no effect on the air conditioning In comparison with normal subjects those with vasomotor rhinitis had an increased enthalpy of the air while the same enthalpy factor was reduced in cases with atrophic rhinitis Laryngectomized patients had no significant difference in the air cond tioning capacity of the nose in relation to normal subjects while patients operated with partial maxillectomy had a considerable reduction in vaporization and total enthalpy

Two of the most important functions of th nose are to warm and to humidify the inspiratory air during its passage through the nose This air conditioning is of the greatest importance for the protection of the mucosa in the lower respiratory tract Measurement of th capacity of the nose to warm and humidif would therefore seem to be needed rathe often in clinical practice, the humidifyin capacity must be especially important It i thus surprising to find that of all various test which have been elaborated for the study c the various nasal functions, there is so far n single one which can be applied clinically t the study of the air conditioning capacity o the nose Those few methods that have bee described are either too complicated for rou tine use, or cause the patient too much dis comfort in clinical practice

Direct measurements of the nasal secretio have been performed by placing a small cellu lose acetate paper strip on the middle par of the nasal septum where it remained fo 10 min and was subsequently weighed (Melon 1968) This method, which was used in ar extensive investigation in which various phar macological drugs were also tested gave many interesting results, but since the air condition ing capacity and the amount of secretion col ted in a small area during a 10 min period a ot synonymous this test has considerable limit ns when used as an air conditioning test

Th air conditioning effect of the nose has pr ipally been investigated in two different ways Firstly, by measurement of the ai

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temperature and humidity in different regions of the upper inspiratory tract at a normal air flow through the nose, and secondly by estimating the capacity of the nose to warm and humidify the inspiratory air when the nose is stressed beyond its normal ability. The first test is thus suitable for studying the effect of changes in the ambient temperature and humidity on the air conditioning at normal air flow and normal conditions in the nose, while the latter is a suitable test for studying the capacity of the nose, with or without pathology, to warm and humidify inspired air when the efforts of the nose are increased above the normal range. In the latter test the nose is thus exerted to a degree beyond that used during normal respiration in a normal climate, for example during breathing of extremely dry air or during breathing with an increased nasal air flow, e.g. during physical exercise.

Measurements of air temperature and humidity in the nose have been performed in some investigations by taking air samples from different parts of the nose or by placing thermometers (Cole, 1953), thermoelements (Seely, 1940, Moe, 1941, Girgis et al. 1974*a*) or thermistors (Cole, 1954) in different sites in the nose or nasopharynx. Girgis et al. (1974*a*) used the heat gain of the inspired air as a measurement of the nasal circulation. By using a thermoelectric micropsychometer which was introduced into the subglottic space, Ingelstedt (1956) managed to record the air temperature and humidity in this region under various conditions, e.g. nasal and oral breathing, room climate and cold chamber.

The effect of dry air on the mucociliary transport in the nasal mucosa has been studied in several papers. *In vitro* studies performed on tracheal specimens from rabbits have shown a close correlation between decreasing air humidity and decreasing mucociliary activity (Mercke et al., 1974). *In vivo* studies have given somewhat contradictory results. Ewert (1965) found by using microscopy of the anterior part of the septum in a series of subjects investigated under varying air humidity, a similar correlation between air humidity and mucus transport, but each subject was investigated once only. Andersen et al. (1972) showed, by using radioactive particles as an indicator of the nasal mucus flow and investigating each subject at least twice at different air humidities, that the nasal mucus flow *in vivo* was not in the least influenced by changes in air humidity between 10% and 70% relative humidity. Even exposure for 78 hours in a climate chamber with very dry air (9% rh) had no effect on the mucus flow (Andersen et al., 1974). The explanation for the inconsistency in the results between *in vitro* and *in vivo* experiments is probably that the reserve humidifying capacity of the normal nasal mucosa is sufficiently high to keep the air humidity in the nasal cavity above the level at which the mucociliary transport is disturbed (Ingelstedt & Ivstam, 1951, Mercke, 1976). Since the most anterior part of the septum is more strongly influenced by a low air humidity than the rest of the nose, it would be of interest to elaborate a method in which the ability of the nasal mucosa to humidify air is strained somewhat more than normally, in order to investigate this humidifying capacity.

Ingelstedt & Ivstam (1951) described a method modified from Aschenbrandt (1886) and Kayser (1887) for measuring the humidifying capacity of the nose. A current of dry air with a flow of 8 l/min was blown into one nostril and out through the other while the soft palate was pressed passively against the posterior pharyngeal wall after local anaesthesia in the pharynx. A psychrometer was placed in the nostril from which the air issued. The air flow of 8 l/min, i.e. twice the normal inspiratory air flow of 4 l/min through each nasal cavity, was chosen because it was necessary in order to get reliable psychrometer readings. When the measurements were continued for 8 min or more it was found that the humidification decreased in the latter period. Furthermore it was found that the humidification was reduced in cases of atrophic rhinitis with extensive crust formation,

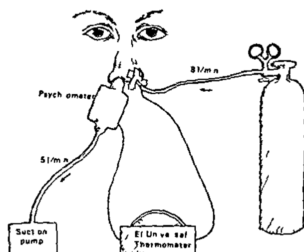


Fig 1 Method for measurement of the air conditioning capacity of the nose Completely dry air passes from a cylinder through a tube into the nasopharynx while the nostril of this side is blocked (usually by the finger of the subject) A thermoelement is placed in this tube close to its opening into nasopharynx The air flow from the cylinder is adjusted to 8 litre/min A psychrometer with a wet and a dry thermocouple and covered by a teflon envelope with a detachable nose piece is introduced into the other nostril A suction pump is connected by a tube to this envelope and 5 litre/min is sucked from this nasal cavity Results from all three thermoelements are measured by an electric Universal Thermometer

but not in those without crusts and it also decreased after subcutaneous injection of 1 mg atropine It is well known that considerable discomfort is evoked when the soft palate is pressed passively against the posterior pharyngeal wall Gagging and profuse salivation are common and not only cause the subject trouble but also seem to increase the nasal secretion Since the air passes in through one nasal cavity and out through the other its passage through the nose is twice as long as usual It is also impossible to investigate each nasal cavity separately

The purpose of the present investigation was to develop a simple clinically practicable method for evaluating the warming and humidifying capacity of the nasal cavities The errors inherent in the method must not of course, render the method unreliable and it must permit measurement of each nasal cavity separately

Another purpose of investigation was t test the method in a clinical series of norm subjects before and after administration (drugs which may be assumed to diminish (increase the nasal secretion The condition capacity should also be investigated in path logical cases, for example, atrophic rhinit vasomotor rhinitis and in patients operat with a partial maxillectomy or with tot laryngectomy

METHOD

After testing several different procedures it b came obvious that procedures involving pus ing of the soft palate posteriorly or obstructi the space between the soft palate and posteri pharyngeal wall with some kind of prosthesis had to be avoided due to the discomfort of gagging and salivation and subsequently in creased nasal secretion A method was therefore elaborated in which air was sucked out from one nasal cavity at a certain air flow while a dry air current was blown through a catheter into the nasopharynx This latter air current had a higher air flow than that sucked out through the investigated nasal cavity (Fig 1) The greater volume of the inflowing air compared with the outflowing implied that the air excess issued from the nasopharynx downwards to the pharynx, thereby at least partly preventing the expiratory air from interfering with the test procedure proceeding in the nose (Fig 2) The subject breathed through the mouth during the entire procedure

The principle of the method is given in Fig 1 Dry air (<0 02 g water/m³) from an air cylinder passed through a regulating valve which was adjusted to 8 l/min through a small silicone catheter which was introduced through one nostril into the nasopharynx This nasal cavity was anaesthetized with Xylocain® spray immediately before the test A small thermoelement (Standard thermocouple probe type F6 Ellab Copenhagen) was placed inside the silicone catheter close to its end A

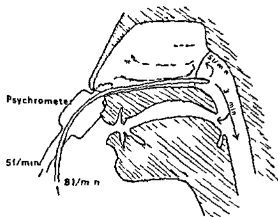


Fig 2 Schematic illustration of the nasal cavity during measuring of the air conditioning capacity Eight litres/min are blown into the nasopharynx while 5 litres are sucked out through the other nostril The additional 3 litres/min pass continuously downwards into the pharynx A thermoelement is placed at the end of the tube for incoming air and a psychrometer to the other nostril The subjects are breathing through the mouth

psychrometer consisting of a dry and a wet thermoelement was connected to the other nostril This psychrometer was modified from one prefabricated psychrometer (type B 19 Ellab Copenhagen) The metal box and fan of the original model were removed and the thermoelements were placed in a Teflon envelope with a nose piece fitting the nostril A pump sucked an airflow of 5 l/min from the investigated nasal cavity through the psychrometer

The response time of the psychrometer was a few seconds when working with the original fan Measurements of the air flow produced by this fan showed that it was 5 l/min and therefore the pump in the experiments was adjusted to 5 l/min The psychrometer was calibrated from the manufacturer to work just at 5 l/min, but control experiments showed that variations of the air flow between 2 l/min and 7 l/min had only a very small influence upon the results when using a constant air flow

Both the psychrometer and the thermoelement in the silicone catheter were connected to an electric Universal Thermometer (type TE3, Ellab, Copenhagen) The dry and wet temperature results were transformed to vapour pressure and to absolute humidity by a nomogram

In a few tests, where prewarmed air was used for the inblowing current, this air passed through a thermostated water bath before reaching the catheter

The heat content in the air and water vapour is usually expressed as enthalpy Calculation of the change in enthalpy (Δi) was done according to the equation (Mollier, 1929)

$$\Delta i = 1.00\,\Delta t + x\,(2500 + 1.86\,t)\ \text{kJ/kg air},$$

where Δt is the increase in temperature of the dry air, x the amount of water vapour in kg/kg dry air, t the dry temperature of the air issuing from the catheter in the nasopharynx The equation is based on the fact that the total increase in enthalpy is the sum of the enthalpy of the air and of the humidity, in which the latter is composed partly of the increasing temperature and partly of the heat required for vaporization The specific heat of air is 1 00 kJ/kg °C, the specific heat of vapour is 1 86 kJ/kg °C and the vaporization heat at 0°C is 2 500 kJ/kg

Test procedure

The subject was sitting in a chair during the test One nasal cavity, usually the more obstructed one was sprayed with 4% Xylocain® Astra without adrenalin and the silicone catheter with the attached thermoelement was introduced 7 cm into the nose which was the length required to reach the choana in adults (Fig 2) The nostril with the catheter was blocked by one of the subject s fingers The nosepiece of the psychrometer was introduced into the other nostril at the moment when the air flows were switched on The wider nasal cavity was usually investigated in order to avoid obstructions for the air flow The inflow in nasopharynx was 8 l/min and the outflow through the other nostril 5 l/min The subject breathed quietly through the mouth during the whole test and was asked to avoid talking or swallowing It was checked that the nose piece fitted hermetically in the nostril Meas

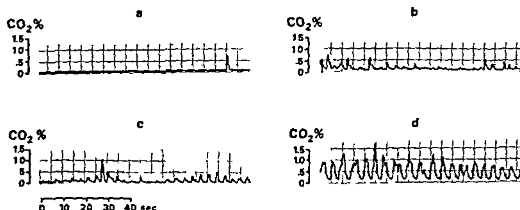


Fig 3 Recording of the carbon dioxide content at the psychrometer by a Medical Gas Analyzer on four different occasions during measurement of the air conditioning capacity of the nose Calibration was performed in such a way that zero corresponded to the carbon dioxide content of the air blown into the nose No variations connected with respiration in Fig 3*a* while Fig 3*b* *c* and *d* show such variations due to intermingling with expiratory air The mean carbon dioxide content measured over a 10 sec period was 0 005% 0 03% 0 05% and 0 6% in *a* *b* *c* and *d* respectively Measurements of the carbon dioxide content in the expired air showed that it was about 4% after 4 sec which means that the corresponding error due to this intermingling amounted to 0 1% 0 8% 1.3% and 15%

urements of the dry and wet temperature of the psychrometer were performed every 30 sec over a period of 10 min, which was the duration of each experiment Measurements of the temperature of the air leaving the catheter in the nasopharynx were carried out after 1, 5, and 10 min

Two or three measurements were performed in several subjects, but only one measurement each day The second or third investigation was made after nasal or subcutaneous administration of various drugs atropine, homatropine, pilocarpine or oximetazoline

Reliability of the method

Respiration through the mouth does not prevent expired air from reaching the nasopharynx By blowing an air current of 8 l/min into the nasopharynx and sucking 5 l/min through one nostril while the other is closed a continuous airstream of 3 l/min will arise, passing downwards from nasopharynx to pharynx, preventing expired air from reaching nasopharynx However there will still be an error when the nasal region is not completely separated from the pharynx Investigations in laryngectomized patients excluded this error In order to measure the error in other persons a tracer is required which shows the intermingling with air from the lower airways It seems logical to use CO_2 as such a tracer, since it is constantly present in the expired air, but only in very low concentrations in ordinary air A continuous recording of the carbon dioxide content in the air sucked through the psychrometer shows the magnitude of the error A newly introduced device, Medical Gas Analyzer 1100 (Perkin Elmer Corp , California) was suitable for that purpose This analyser uses the principle of mass spectrometry It takes an air sample of 1 ml/sec through a capillary tube and measures dynamically and essentially instantaneously up to eight components of the sample

Continuous measurements of CO_2 from the tube of the psychrometer under circumstances simulating an ordinary experiment were done on four different occasions (Fig 3) The rise in CO_2 content was in one experiment as low as 0 005% and it had in this experiment a constant level (Fig 3*a*) while in the other three experiments there were elevations during each expiration The mean increases in CO_2 in these three experiments measured over a period of 10 sec were 0 03% 0 05% and 0 6% Measurements of the CO_2 content in the

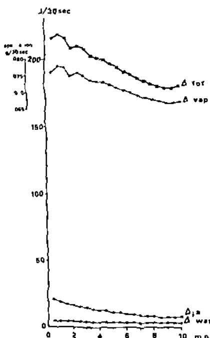


Fig 4 The enthalpy factors during an experiment for measuring the air conditioning capacity of the nose Δi_{tot}=increase in total enthalpy Δi_{air}=increase in enthalpy of the dry air Δi_{vap}=enthalpy due to vaporization of water vapour and Δi_{wat}=increase in enthalpy caused by the raised temperature of the water vapour The vaporization which dominates among the separate enthalpy factors can also be read in g H_2O for each 30 sec period

expired air in these subject showed that it was about 4% after 4 sec, which is the usual duration of a breath Based on these values the error in these four experiments were 0 1% 0 8% 1 3% and 15% The lower values were obtained in well cooperating persons accustomed to the procedure from several previous investigations It seems likely that the maximum error due to the intermingling with expired air in this method is thus about 15%, but usually smaller It also shows that a complete elimination of the expired air may occur, as in Fig 3*a*

MATERIAL

Altogether 38 persons were investigated and they took part in 68 experiments Among these persons there were 22 normal subjects 4 with vasomotor rhinitis, 4 with atrophic rhinitis, 4 laryngectomized and 4 operated with partial maxillectomy The ages of these 38 persons ranged between 17 and 80 years with a mean of 48 years

RESULTS

The different enthalpy factors in normal subjects

The total increase in enthalpy (Δi_{tot}) of the air during its passage through the nasal cavity consists, as shown in the equations given when describing the method, of three different factors increase in enthalpy of the dry air (Δi_{air}), vaporization of water (Δi_{vap}) and increase in enthalpy of water vapour (Δi_{wat}) Fig 4 shows these three factors and Δi_{tot} each 30 sec period during an experiment Vaporization dominates among these factors In order to see the correspondence between enthalpy for vaporization and the quantity of vaporized water the latter is also given on the abscissa A slightly decreasing tendency during the course is seen in Fig 4 but other experiments had a flat or slightly increasing tendency No correlation was found between the course of the enthalpy recording and the diagnosis of the subject or any administered drug

In 19 normal subjects the total increase in enthalpy of the 50 litres air passing the nasal cavity during a 10 min period (Δi_{tot}) was as a mean 4418 J, while the corresponding values for Δi_{air} Δi_{vap}, and Δi_{wat} were 368 J, 3956 J and 94 J respectively This corresponds to a vaporization of 1 58 g H_2O during the experimental period The air was usually almost saturated with water vapour when reaching the psychrometer

In 3 other normal subjects experiments were performed using air which was preheated to a temperature of 33 0°C when reaching the nasopharynx and this was done by a water bath thermostated to 44 3°C between the air cylinder and the tube going to nasopharynx The normal temperature of the nasal mucosa is about 33°C (Drettner, 1961) The mean

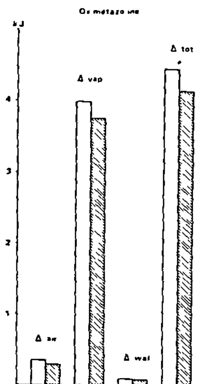


Fig 5 Increase in enthalpy during a 10 min experiment performed with and without 3 drops oximetazoline (Nezeril® Draco) in the nose For explanations see Fig 4 The Δt_{tot} differed significantly between the two occasions □=Normal subjects without any drug ▨=The same subjects after oximetazoline

values of Δt_{tot}, Δt_{air}, Δt_{vap} and Δt_{wat} in [th]e 3 subjects were 4 102 J, 0 J, 4 007 J and [illegible] J, respectively These correspond very closely to the values in the experiments without preheating except for the elimination of Δt_{air}

Pharmacological effects in normal subjects

The following pharmacological substances were tested in normal subjects

(1) local administration of 3 drops of 2% pilocarpine in one nasal cavity

(2) local administration of 3 drops oximetazoline 0 25 mg/ml (Nezeril® Draco) in one nasal cavity,

(3) local administration of 3 drops of 1% homatropine in one nasal cavity

(4) subcutaneous injection of 0 5 mg atropine

The nasal administration was given about 15 min before the experiment, while the subcutaneous injection was given 20–30 min before the measurement The subjects receiving atropine subcutaneously were mostly those who were to be tonsillectomized and the atropine was given as premedication for operation All subjects who received drugs were also measured without any drug, one or several days earlier

Pilocarpine gave inconsistent results increase of Δt_{tot} in 2 and decrease in 2 subjects in comparison with control experiments in the same subjects

Oximetazoline (Fig 5) gave a decrease of Δt_{tot} in all 5 subjects and this decrease was significant ($p<0\ 05$) The decreases, calculated in per cent of each enthalpy factor were 18%, 6% and 10% for Δt_{air}, Δt_{vap} and Δt_{wat}, respectively The vaporization during the 10 min period was, as a mean, 1 50 g H_2O after oximetazoline

Homatropine had no significant effect Δt_{tot} increased in 4 and decreased in 2 of the 6 investigated subjects

Atropine subcutaneously (Fig 6) gave a decrease of Δt_{tot} in all 5 investigated subjects and this decrease was statistically significant ($p<0\ 05$) and so was also that for Δt_{wat} ($p<0\ 05$) The decreases, expressed in per cent for Δt_{air}, Δt_{vap} and Δt_{wat}, were 19%, 10% and 15%, respectively The vaporization during the experiment after atropine was 1 40 g as a mean

Nasal air conditioning in pathological cases

The 4 investigated patients with *vasomotor rhinitis* had an Δt_{tot} which, as a mean was 4 453 J, i e somewhat (though not significantly) greater than that in normal subjects (Fig 7) Of the three enthalpy factors, Δt_{air} was significantly higher than normal ($p<0\ 05$) while the other did not differ significantly The vaporization during 10 min was 1 57 g as a mean

Four patients with *atrophic rhinitis* were

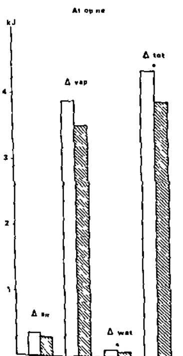


Fig 6 Increase in enthalpy with and without sub cutaneous injection of 0 5 mg atropine Significant dif ference for Δi_{wat} and Δi_{tot} □=Normal subjects without any drug □=The same subjects after atropine

examined They were all almost free from crusts when investigated They had an Δi_{tot} of 3772 J as a mean, which was smaller (though not significantly so) compared with the normal subjects In this group too Δi_{air} differed significantly ($p<0\ 05$) from that in normal subjects those with atrophic rhinitis having the lower value The other two enthalpy factors did not differ significantly from those in the healthy subjects The mean vaporization during the experiments was 1 34 g H_2O

Four *laryngectomized patients* were studied They had been operated at least 6 weeks before the investigation Their mean Δi_{tot} was 4 610 J, which was higher than among the normal subjects, though the difference was not significant Neither did any of the separate enthalpy factors differ significantly from normal values The vaporization was a mean 1 65 g

A special group consisted of patients who were operated with *partial maxillectomy* at least a few months before the experiment Two of these patients had had benign mesenchymal tumours and two malign tumours (adenoid cystic carcinoma, adenocarcinoma) in the posterior part of the nose extirpated by removal of the lateral nasal wall but with preservation of the palate The patient with adenocarcinoma had received irradiation 7 years before the experiment, while the rest had not Their mean Δi_{tot}, which was 3 444 J, was significantly lower than among normal subjects ($p<0\ 001$) The same degree of significance was found for both Δi_{vap} and Δi_{wat}, while Δi_{air} did not differ significantly The amount of vaporized water was, as a mean, 1 25 g

DISCUSSION

The lack of a clinical method for studying the air conditioning capacity of the nose is striking, when one realizes how common nasal symptoms such as dryness of the nose or running nose are It is also in contrast to the great number of methods described for investigation of another common nasal symptom, namely nasal obstruction The only method which in a technically reliable way measures the air conditioning ability of the upper respiratory tract is that of Ingelstedt (1956) who used a subglottic psychrometer However, it is not possible to use this method in clinical practice, as it involves a puncture of the crico thyroid membrane when introducing the psychrometer

All methods which involve pushing of the palate posteriorly or the introduction of some prosthesis between the soft palate and the posterior pharyngeal wall cause considerable discomfort and probably also an increased salivation and nasal secretion

The method of Melon (1968) using paper strips on the nasal septum is very simple and does not cause any discomfort One disadvantage is that it measures only a very limited area and it seems likely that not only

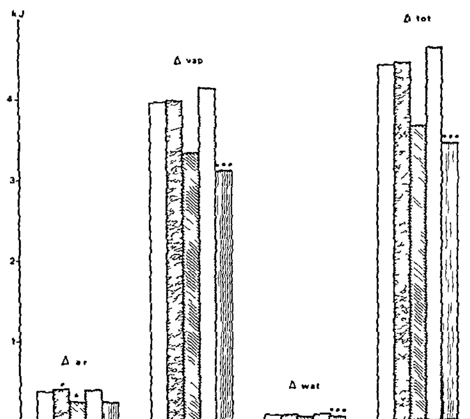


Fig 7 Enthalpy factors in normal subjects in relation to those with vasomotor rhinitis atrophic rhinitis laryngectomy and partial maxillectomy Statistically significant differences compared with normal subjects are shown by asterisks ☐=Normal persons (*n*=22) ☐=Vasomotor rhinitis (*n*=4) ☐=Atrophic rhinitis (*n*=4) ☐=Laryngectomized patients (*n*=4) ☐=Patients with partial maxillectomy (*n*=4)

he secretion under but also around the paper is absorbed To distinguish between secretion present before the test and that produced during the test is also more difficult than with other methods

The method presented here is based on a new principle by introducing into the nasopharynx an air stream which is greater than that sucked out through the nostril The additional air flow passing continuously downwards from nasopharynx to pharynx has when the subject breathes through his mouth the purpose of preventing expired air from the lungs from interfering with the artificial air flow in the nose Measurement of the carbon dioxide content in the air sucked out through the nose showed that the error due to intermingling with expired air varied between 0 1% and 15 Even if the error were to amount to 15% it would be of no practical importance for such a test when used clinically

The subject must be instructed to breath quietly through the mouth to avoid swallowing and to be silent and relaxed in order to keep the error as low as possible The method does not cause any noticeable discomfort and thus probably does not interfere with nasal secretion more than desired One disadvantage with the method is that it gives a retrograde air flow in the nose but this is probably of minor importance when the purpose is to measure the air conditioning capacity of the nose rather than the air conditioning function For that reason the normal regaining of heat and moisture during expiration in the nose (Ingelstedt 1956) is also eliminated in the de

scribed method and furthermore a completely dry air stream is used

With the present method it is possible to measure all three enthalpy factors separately i e the increase of enthalpy of the air and of the water vapour and the vaporization The latter constitutes normally about 90% of the total enthalpy in the way the experiments are performed Prewarming of the air before reaching the nasopharynx was tried in a few experiments The only result was an elimination of the factor Δi_{air}, while the other factors remained unchanged This was thus only a disadvantage, as it reduced the information gained

The normal vaporization during a 10 min experiment amounted to 1 58 g H_2O (mean) Assuming theoretically that the test is continued for 24 hours, this would mean that 228 g H_2O would vaporize from one nasal cavity, or 456 g H_2O from the two, if a bilateral test was performed According to Toremalm (1960) the normal supply of water vapour from the entire upper respiratory tract during 24 hours is about 430 g Since our value represents only the nasal cavities while Toremalm discusses the area from the nostrils to the subglottic region, it is obvious that our method causes a somewhat greater nasal vaporization than that normally found Our method thus implies at least a slight stress on the humidifying capacity of the nose, and the normal condensation during expiration is also eliminated in this method

Two of the administered pharmacological substances gave statistically significant results Oximetazoline gave a decrease in Δi_{tot} which concerned all three enthalpy factors, but especially the warming of air when expressed in per cent Subcutaneous injection of atropine also gave a significant decrease in Δi_{tot} and also in Δi_{wat} and furthermore the vaporization became rather low, 1 40 g H_2O per 10 min This indicates that the effect of oximetazoline is principally related to heat elimination from the nasal mucosa, which is in agreement with the fact that it causes vasoconstriction Atropine has a more pronounced effect on the water vapour, which confirms the well known fact that it inhibits secretion from different glands, among them also those in the nasal mucosa The fact that the vaporization did not differ significantly from that in normal subjects after atropine is probably due to the smallness of the material and the great dispersion for vaporization, while Δi_{wat}, which is actually closely connected with the vaporization had less dispersion Ingelstedt & Ivstam (1951) also reported a decreased humidification after subcutaneous injection of atropine

It is somewhat surprising that local administration of homatropine or pilocarpine had no significant effect on the enthalpy or on the vaporization A local inhibition of these drugs seems unlikely, since an effect is obtained on local administration in the eye and some of the tested subjects had also symptoms from the eyes after nasal administration Atropine and homatropine are known to have a direct effect on the blood vessels in the face, giving a vasodilatation which is not transmitted by the effect of atropine on acetylcholine and such an effect might counteract a decreased nasal secretion in such a way that the total enthalpy does not change, but when analysing the various enthalpy factors it should still have been revealed Pilocarpine acts in the same way as acetylcholine, for example on glands, but no such effect was observed on direct administration in the nose So far, no acceptable explanation for the lack of effect by homatropine and pilocarpine can be presented It is interesting to note that no one has yet suggested the use of these drugs for the inhibition or stimulation of nasal secretion, despite the fact that logically they should have been tried for that purpose at some time even, if it has not been published

In the groups of hy

this method it was found that patients with vasomotor rhinitis had significantly higher Δi_{air} than normal subjects, showing that vasomotor rhinitis has an increased heating capacity in the nose which may be related to a vasodilatation Atrophic rhinitis had instead significantly lower Δi_{air} than in normal subjects, which shows that the air heating is reduced in this disease The production of water vapour during the experiment was also low but not significantly different from the normal value Ingelstedt (1956) found a decreased humidification only in those patients with atrophic rhinitis who had extensive crust formation but not in those without crusts Girgis et al (1974*b*) reported a low heating power of the nose in cases with atrophic rhinitis but it was not reported if crusts were present

A group of laryngectomized patients had a high Δi_{tot} and a high vaporization rate, though not significantly different from that among normal subjects It is worth emphasizing that all intermingling with expiratory air is completely eliminated in laryngectomized patients When the mean value in these patients is thus higher than in normal jects where an intermingling with expired may occur to some extent, thereby raising all enthalpy factors, it confirms the fact that the error due to intermingling in normal persons is scarcely of any importance Histological studies on the nasal mucosa after laryngectomy have demonstrated an increase in goblet cells (Ewert 1965) and the temperature of the nasal mucosa is higher than in persons with nasal respiration (Drettner, 1961) all of which work to raise the total enthalpy

In a group of patients operated on with partial maxillectomy the factors Δi_{tot} Δi_{vap} and Δi_{wat} were greatly decreased statistically It confirms that the humidification is insufficient from such a wide cavity which also at least partly lacks its normal mucosa and is also sometimes covered by crusts

ZUSAMMENFASSUNG

Es wird eine einfache Methode zur Messung der Luftaufbereitungsfähigkeit der Nase vorgestellt Trockene Luft wird mit einem Volumenstrom von 8 l/min durch einen Katheter in den Nasopharynx geleitet während mit einem Volumenstrom von 5 l/min Luft aus der zu untersuchenden Nasenhöhle nach Passage eines Psychrometers abgesaugt werden Die überschüssigen 3 l/min strömen in den Pharynx und vermindern damit die Vermischung mit Ausatmungsluft Unter Verwendung von CO_2 als Indikator zeigte sich eine Fehlerquelle von maximal 15% und oft um 1% Die drei verschiedenen Enthalpiefaktoren Zunahme der Enthalpie der trockenen Luft der Verdampfung und Zunahme der Enthalpie des Wasserdampfes wurden getrennt berechnet wobei die Verdampfung der dominierende Faktor war Die errechnete Gesamtwasserdampfzufuhr zeigte daß die vorgestellte Methode jedenfalls eine geringe Belastung der Befeuchtungsleistung verursacht Pharmakologische Untersuchungen zeigten daß subcutan verabreichtes Atropin die Totalenthalpie und die Enthalpie des Wasserdampfes verringert während die lokale nasale Gabe von Oxymethazolin ebenfalls die Totalenthalpie vermindert Die nasale Verabreichung von Homatropin oder Pilocarpin hat auf die Luftaufbereitungsfähigkeit keinen Einfluß Im Vergleich mit gesunden Untersuchungspersonen zeigen Patienten mit vasomotorischer Rhinitis eine Zunahme der Enthalpie der Luft wogegen derselbe Enthalpiefaktor in Fällen von atropischer Rhinitis vermindert war Laryngektomierte Patienten boten keine signifikanten Unterschiede hinsichtlich der Luftaufbereitungsfähigkeit gegenüber Normalpersonen während Patienten mit Status post partieller Maxillektomie eine beträchtliche Verringerung der Enthalpie der Verdampfung und der Totalenthalpie aufwiesen

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SYMPATHETIC NERVE PATHWAYS TO THE NASAL VASCULATURE OF THE CAT

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(Received December 9 1976)

Abstract The sympathetic nerve pathways to the nasal vasculature were examined in 8 cats by recording the effects of section of the orbital nerves on vasoconstriction produced by cervical sympathetic nerve stimulation No sympathetic fibres were found in the sphenopalatine and infraorbital nerves The majority were conveyed by either the Vidian or ethmoidal nerves the remaining fibres are believed to reach the nasal cavity by the periarterial plexus

A number of investigators have found that the majority of sympathetic fibres innervating the nasal blood vessels of the cat are conveyed by the Vidian nerve (Slome, 1955, Malcomson 1959, Malm 1973 Anggård & Densert, 1974) in the dog the majority of these fibres ...ar to be in the maxillary nerve (Jackson & Rooker 1971 Gadlage et al 1975) Malcomson (1959) was of the opinion that in the cat an insignificant number of vasoconstrictor fibres reach the nose by the ethmoidal nerve and a minute proportion via branches of the maxillary artery

The present investigations were designed to determine the routes taken by sympathetic fibres to reach the nasal vasculature of the cat and to obtain an estimate of the proportion of sympathetic fibres conveyed by these pathways

METHOD

Cats of either sex, 2–4 kg body weight were anaesthetised with pentobarbitone sodium 40 mg/kg i p and the trachea was cannulated.

Vascular changes in the nose were recorded as a pressure change in the sealed nasal cavity by means of a short plastic cannula which was inserted into the nostril and connected to a pressure transducer (Bell & Howell type 4-327 L223) and a Devices M2 pen recorder (Wilson & Yates, 1975) This method only records the overall vascular changes but according to Anggård & Edwall (1974) and Malm (1974) it will mainly reflect the responses in the capacitance vessels of the nasal mucosa.

The peripheral end of the preganglionic cervical sympathetic chain of one side was mounted on bipolar platinum electrodes and covered with small cotton wool plugs soaked in liquid paraffin (B P) The opposite preganglionic cervical sympathetic chain was also sectioned and the ends crushed

The Vidian ethmoidal infraorbital and sphenopalatine nerves were exposed in the orbit of the side to be stimulated by the method of Eccles & Wilson (1973) and loose ligatures placed round them to aid in subsequent section In some experiments the cut peripheral end of the ethmoidal nerve was placed on bipolar platinum electrodes

The cervical sympathetic chain was stimulated by square wave impulses of supramaximal intensity and 0 5 msec duration at frequencies ranging from 0 2 to 15 0 Hz delivered from a Grass S4 stimulator through an isolation unit (SIU4) In order to investigate the

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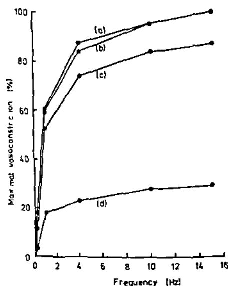


Fig 1 Vasoconstrictor frequency/response curves obtained on supramaximal preganglionic cervical sympathetic stimulation before (*a*) and after section of (*b*) infraorbital and sphenopalatine nerves (*c*) ethmoidal nerve (*d*) Vidian nerve

presence of sympathetic and parasympathetic nerve fibres in the ethmoidal nerve, it was stimulated firstly at supramaximal intensity and 1 0 msec duration and secondly at 2 0 V and 0 2 msec, at frequencies ranging from 0 2 to 15 0 Hz because these stimulation parameters when applied to the Vidian nerve produce vasoconstriction and vasodilation respectively (Eccles & Wilson 1974) In all instances the nerves were stimulated for 15 sec every 3 min

The arterial blood pressure was recorded in all experiments from a femoral artery by means of a pressure transducer (Bell & Howell type 4 327 221) and a pen recorder (Devices M2)

RESULT

In 8 cats the cervical sympathetic chain of one side was stimulated at frequencies of 0 2 1 0 4 0 10 0 and 15 0 Hz and vasoconstriction in the nasal cavity recorded before and after section of the sphenopalatine infraorbital ethmoidal and Vidian nerves

The vasoconstrictor responses were expressed as a percentage of the response obtained at 15 0 Hz before nerve section in each cat The percentage reduction in the response to stimulation at 15 0 Hz after section of each nerve was used as an index of the percentage of the total number of sympathetic fibres conveyed by that nerve to the nasal vasculature The percentage reductions at the lower frequencies were similar to those at 15 0 Hz

In the experiment shown in Fig 1, no sympathetic fibres were found in the sphenopalatine and infraorbital nerves whereas the ethmoidal nerve contained approximately 13% and the Vidian nerve 57% Approximately 30% of the sympathetic fibres were unaccounted for but it is likely that the remaining responses were due to sympathetic fibres conveyed by the arterial supply to the nose

The percentage of the total number of sympathetic fibres which were conveyed by the Vidian and ethmoidal nerves and periarterial plexus in 8 cats are shown in Table I In none of the experiments were sympathetic fibres detected in the infraorbital and sphenopalatine nerves

Stimulation of the ethmoidal nerve in 7 cats at a supramaximal intensity and 1 0 msec duration evoked vasoconstriction with a frequency/response curve similar to that shown in Fig 1*a* Stimulation with parameters which evoke vasodilation in the Vidian nerve (2 0 V and 0 2 msec) produced no response in 4 and

Table I *The percentage of the total sympathetic fibres conveyed to the nasal vasculature by different pathways in 8 cats*

IF & S P N Infraorbital and sphenopalatine nerves

Cat no	% of sympathetic fibres conveyed by each pathway: Vidian *n*	Ethmoidal *n*	Periarterial plexus	IF & S P N
1	82	16	2	0
2	57	13	30	0
3	52	26	22	0
4	10	77	13	0
5	11	71	18	0
6	60	12	28	0
7	0	79	21	0
8	53	23	24	0

vasoconstriction in 3 cats which amounted to between 10 and 45% of the response evoked at each frequency on supramaximal stimulation

DISCUSSION

The results of these investigations indicate that in the cat sympathetic vasoconstrictor fibres are conveyed to the nasal vasculature in varying proportions by the ethmoidal and Vidian nerve and the arterial supply to the nose No sympathetic fibres were found in the infraorbital or sphenopalatine nerves which is in contrast to the situation in the dog (Jackson & Rooker, 1971) Gadlage et al (1975) later produced evidence to show that the presence of sympathetic fibres in the maxillary nerve of the dog represented a species difference

Although in the majority of the cats the greater proportion of fibres were in the Vidian nerve, there was considerable inter-individual variation with the ethmoidal nerve which in 3 cats contained between 70 and 80% of the total number of sympathetic fibres

In one animal Vidian nerve section produced no reduction in the vasoconstrictor responses indicating that no sympathetic fibres are contained in the nerve (Table I) A similar bservation was recorded in one cat by Malm (1973)

The findings indicate that in some cats the proportion of vasoconstrictor fibres present in the ethmoidal nerve is by no means as small as Malcomson (1959) speculated This also applies to the number of sympathetic fibres conveyed by the arterial supply, which in 8 animals ranged from 2 to 30%

Vasoconstriction recorded as a result of supramaximal ethmoidal nerve stimulation confirmed the presence of vasoconstrictor fibres in this nerve, although stimulation at 2 0 V 0 2 msec , parameters which when applied to the Vidian nerve produce vasodilation (Eccles & Wilson, 1974) evoked either a small vasoconstrictor response or none at all The investigations suggest that vasoconstrictor but not vasodilator fibres are contained in the ethmoidal nerve, a finding, which is in contrast to the histological studies of Nomura & Matsuura (1972) who identified cholinergic fibres in this nerve in man

ZUSAMMENFASSUNG

Die sympathischen Innervationswege der Nasenblutgefäße wurden an acht Katzen untersucht indem die durch Reizung des Halssympathikus hervorgerufene Vasokonstriktion vor und nach Durchschneidung der Nerven in der Augenhohle gemessen wurde Keine sympathischen Fasern wurden im Nervus sphenopalatinus und infraorbitalis gefunden Während die meisten Fasern entweder im Nervus Vidianus oder ethmoidalis verliefen deuten die Versuche darauf hin, daß die restlichen Nasenblutgefäße uber die perivaskularen Geflechte erreichen

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STUDIES ON THE ALLERGEN-CHALLENGED HUMAN NASAL MUCOSA

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(Received October 30 1976)

Abstract Contrary to the results reported on experimental anaphylaxis in animals we could not discover any effect of intranasal allergen challenge on ciliary function epithelial structures or subepithelial number of mast cells in 28 hay fever patients

It is of importance for our comprehension of allergic airway diseases to know how an acute attack of pollinosis affects the human nasal mucosa In investigations in sensitized guinea pigs and rabbits, drastic changes in the mucosa following allergen challenge have been demonstrated Strömme (1957) showed that pollen grains of birch and rye could penetrate into the mucosa of guinea pigs, sensitized as well as unsensitized On the other hand Chevance (1957 and 1971) and Naumann (1959) found that penetration only took place when the animal was sensitized against the actual pollen All three authors have demonstrated pronounced changes in the nasal mucosa following pollen challenge The finding that early cilia paralysis and destruction took place around the pollen grain, followed by destruction of epithelial cells and pollen penetration into the mucosa (Table I), was of the greatest interest The purpose of the present study was to investigate whether these early epithelial changes, described in animals, have any importance for human hay fever

Table I *Scheme of changes in the nasal mucosa of animals after challenge (Chevance, 1971)*

1st – 10th min	Cessation of ciliary beating
10th – 30th min	Destruction of ciliary and epithelial cells Penetration of pollen
30th min – 48 hour	Appearance of eosinophil cells
2nd – 10th day	Appearance of macrophages
15th – 27th day	Appearance of plasma cells

MATERIAL AND METHODS

Twenty-eight subjects (12 women, 16 men) with a typical history of seasonal hay fever caused by grass, lasting for at least 3 years, participated in the investigation Their ages ranged from 14 to 44 years None of the patients had perennial rhinitis and none had previously received immunotherapy In all cases the history was confirmed by positive skin testing with timothy pollen extract The study was carried out in January and February

In 18 subjects a biopsy was taken without anaesthesia from the lower edge of the inferior turbinate, about 1 cm posterior to the front edge By use of a micropipette 2000–6000 timothy pollen grains were applied to the same spot in the contralateral half of the nasal cavity Within a few minutes this elicited typical symptoms of hay fever in all the patients Thirty minutes later when most of the symptoms had ceased a new biopsy of the chal-

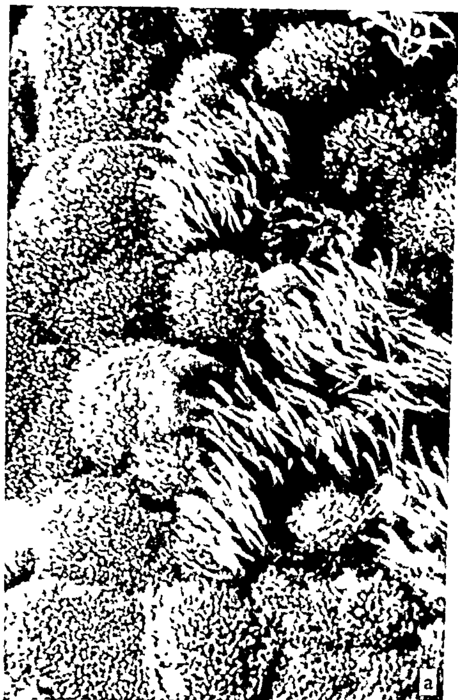
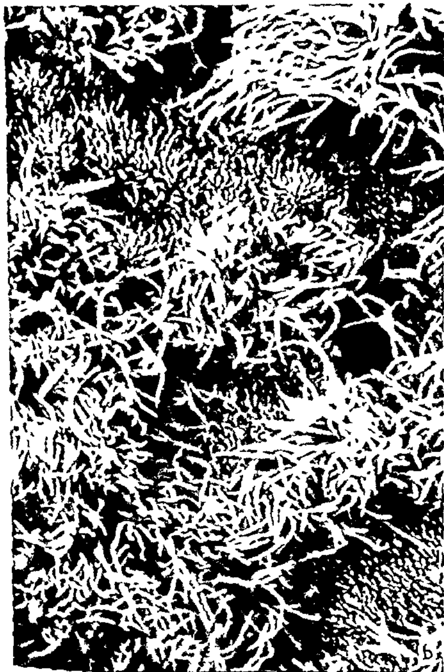


Fig 1 Representative scanning electron micrographs of nasal mucosa before (*a*) and after (*b*) allergen challenge There is no bulging of epithelial cells dilated intercellular spaces or pathology of cilia and microvilli (×3000)

lenged mucosa was taken and coded for blind examination in the scanning electron microscope and the light microscope

The biopsy specimens were fixed in glutaraldehyde critical point dried and prepared for scanning electron microscopy After examination the same specimens were embedded in Epon and sectioned for light microscopy as described earlier (Mygind 1975)

In 10 subjects the nasal mucociliary transport time was determined before and 30 minutes after allergen challenge of the anterior part of the inferior turbinate For measuring the mucociliary transport time the saccharin



technique described by Andersen et al (1974) was used A saccharin dye particle was placed on the upper part of the inferior turbinate at a distance of 4 cm from the tip of the nose and the patient was then asked to swallow once per minute The period until the patient could feel a sweet taste was the nasal mucociliary transport time The appearance of a blue spot in the throat could verify the patient s statement

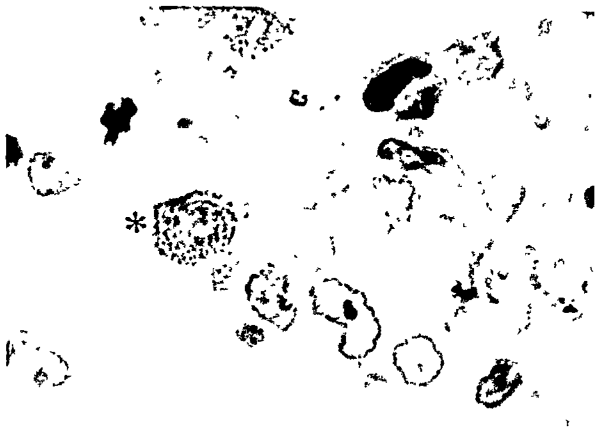


Fig 2 In toluidine blue stained sections mast cell granules are distinct and the cells easily recognized and counted (×280)

RESULTS

The biopsies had a size of 3–5 mm² and all had an undamaged central surface suitable for microscopy On an average 10% of the epithelial surface was covered by cilia

When the coded pairs of specimens were examined it was not possible to judge whether a biopsy had been taken before or after allergen challenge In the scanning electron microscope attention was given to the amount of mucous secretion dilated intercellular spaces, bulging of cells and appearance of cilia and microvilli In the light microscope attention was focused on appearance of epithelium and basement membrane cell infiltration in epithelium and in lamina propria tissue oedema and blood vessels

Nor did the subsequent detailed study of four representative scanning electron micrographs from each specimen disclose an microvilli or cilia pathology (Fig 1) In th light microscope mast cells were distinctiv and easy to identify and count (Fig 2) Th average number of subepithelial mast cells ha not changed significantly by the allergen cha lenge (Fig 3) Nor was the number o eosinophils in the subepithelial layer changec

A final examination after breaking the cod suggested an increased number of perivascula eosinophils and slightly dilated blood vessel in the post challenge biopsies A thorough ex amination of 16 sections from each post chal lenge biopsy did not reveal any pollen lik structures nor did the scanning electro microscopy

The average (mean) nasal mucociliary trans port time for the 10 subjects was 11 minute before as well as after allergen challenge

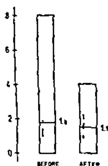


Fig 3 Number of mast cells in submucosa per high power field (×800) (mean of 10 fields)

of nasal blood flow will follow intranasal allergen challenge (Ingelstedt & Rundcrantz, 1964) The vascular changes observed by Naumann are probably caused by platelet activating factor (PAF) PAF is released from allergen challenged mast cells is capable of inducing vascular pathology, and is shown to be an important mediator of immediate allergic reactions in rabbits (Benveniste et al , 1972) while it is certainly of less significance for human allergic disease (Austen et al 1975)

The biological features described may explain in part the discrepancies between *in vivo* animal and human studies, but we have no explanation of the fact that pollen stops ciliary beating also *in vitro* in sensitized mucous membranes, as demonstrated by Chevance (1957, 1971) However, from our point of view it is sufficient to conclude that pollen induced cilia paralysis, destruction of cilia and epithelium and pollen penetration of the mucosa during the first 30 minutes after challenge is apparently of no importance in human disease

DISCUSSION

In hay fever patients we could not reveal any signs of destruction of cilia or epithelium impaired ciliary function or pollen penetration of the mucosa following allergen challenge Thus we were unable to confirm the results of animal experiments (Chevance, 1957 1971, Naumann 1959, Stromme, 1957) in man

It is necessary to take into consideration the different methods used, and the difficulties and uncertainties connected with studies on humans While large mucosal surfaces can be examined *in vivo* and *in vitro* in animals we can only obtain small human tissue biopsies from the anterior part of the nose In animals exact methods are available for measurement of the ciliary function Our measurement of human mucociliary function may have been influenced by the increased amount of watery secretion following allergen challenge Although it is difficult to assess the significance of methodological differences we find it highly unlikely that such factors can explain the totally different results obtained in the animal experiments and in our study on humans

It has been demonstrated convincingly by Naumann (1961) that nasal allergen challenge of sensitized animals is followed by pronounced vascular disturbances (thrombosis, bleeding coagulation) These changes will certainly result in impaired mucosal vitality There is no evidence to suggest that this also happens in man although some disturbances

In guinea pigs the number of nasal mast cells was reduced to less than 50% following allergen challenge (Boreus, 1961) But in hay fever patients we could not discover any significant quantitative changes in subepithelial mast cells after a single challenge This is in agreement with the known differences in the course of animal anaphylaxis and human disease After a few severe reactions the animal may become desensitized, probably due to lack of reactive mast cells while a patient is able to react every day for an unlimited period, this indicates the presence of reactive mast cells As it takes weeks to build up a new mast cell population it can be concluded that a single allergic reaction in man only releases a small quantity of the chemical mediators available in the mucous membrane It is possible that prolonged and severe reactions may deplete this histamine depots the bronchial mucosa of patients who died in status asthmaticus showed a considerable reduction in the number of mast cells (Conell, 1971)

With regard to cilia as well as mast cells the

present study emphasizes the differences between experimental animal anaphylaxis and human allergic disease

ZUSAMMENFASSUNG

Im Gegensatz zu Angaben in der Literatur uber experimentelle Anaphylaxie bei Tieren konnte in dieser Studie bei 28 Heuschnupfen patienten nach intranasaler Allergenbelastung keinerlei Einwirkung auf die Zilienfunktion, die Epithelstruktur oder die Anzahl von subepithelialen Mastzellen festgestellt werden

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DISODIUM CROMOGLYCATE NASAL SPRAY IN THE TREATMENT OF PERENNIAL RHINITIS

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Abstract In a double blind crossover trial 24 patients with perennial rhinitis have been treated with 2% disodium cromoglycate (DSCG) nasal spray 15 patients preferred DSCG to placebo 9 patients showed no preference Analysis of the diary cards showed a significant preference of DSCG in the group which received placebo first for the symptoms blocking secretion and sneezing ($p<0.1$) while no preference between the treatment periods occurred *in the group receiving DSCG first* A significantly higher IgG concentration in serum was found in 15 patients with preference for DSCG than with the remainder ($p<0.05$) No other changes in immunoglobulin concentration in nasal secretion or serum were found which could be related to the effect of DSCG DSCG nasal spray is considered a valuable supplement in the treatment of perennial rhinitis

Disodium cromoglycate (DSCG) has been shown to be effective in the treatment of hay fever in different formulations (Holopainen et al, 1971, Illum et al, 1973) Engstrom (1971) has shown that DSCG has a protective effect on the nasal mucosa when this is exposed to specific antigen in pollen sensitive patients

In perennial rhinitis, DSCG has also been found to be effective (Holopainen et al 1973, 1975 Mygind et al, 1972) However in this condition the pathological picture is more complex than in hay fever There are many difficulties in differential diagnosis and the results of clinical trials are similarly difficult to interpret In order to overcome these problems Thorne & Bradbeer (1972) and Girard & Bertrand (1975) used rhinomanometry as an objective criterion in the evaluation of DSCG in the treatment of perennial rhinitis Also Brain et al (1974) have shown in electron microscopic studies of nasal biopsies before and after treatment that DSCG protects the mast cell against degranulation and reduces the inflammatory reaction in the mucosa

DSCG (Lomudal®) is now available in a 2% aqueous solution delivered by a novel metered dose spray This spray does not use propellant gases and delivers a consistent dose (2.6 mg/spray) We have carried out a clinical trial of this formulation of DSCG in perennial rhinitis and in addition tried to evaluate the effect in relation to the immunoglobulin concentration in serum and nasal secretions before treatment

MATERIAL AND METHODS

Twenty five patients were included in the trial All had had non seasonal vasomotor rhinitis for at least 2 years which required treatment Patients with nasal polyps of such a size that they caused significant obstruction were not included nor were patients with asthma needing treatment, nor those with signs of active sinusitis Previous treatment with steroids or hyposensitization were not contra indications provided that the patients still had significant symptoms

The trial was carried out on a double blind crossover basis with all patients commencing

Table I *Patient preferences*

Patient group	Number of preferences for DSCG	Placebo	Neither
DSCG–Placebo	9	0	3
Placebo–DSCG	6	0	6
All patients	15	0	9

on 1st February, 1975 Patients were randomly allocated to either DSCG or placebo for 4 weeks and then transferred to the alternative treatment

At the first visit a full ENT examination was carried out in order to assess nasal polyps and septum deviation Nasal smears were taken for eosinophil examination Sinus X-rays were carried out and if the maxillary sinuses appeared opaque a diagnostic maxillary puncture was carried out Skin tests against a number of antigens were performed by intracutaneous testing Nasal secretion was obtained for quantitative determination of IgG, IgA, IgM, IgE and albumin according to the method of Lonn et al (1972) Serum levels of immuno globulins were estimated (to be published Illum & Balle)

Each treatment was given for 4 weeks the osage being one spray (2 6 mg) to each nostril) times a day Antihistamine tablets were prescribed to be used as required Diary cards were issued for daily recording of the symptoms blockage running and sneezing on a semi quantitative scale from 0 to 3 The use of antihistamine tablets was also recorded

Examinations were carried out after 4 and 8 weeks in order to evaluate clinically the severity of the symptoms Eosinophils in a nasal smear evaluation were also estimated

At the end of the trial the patients were asked which treatment they preferred and about possible side effects

RESULTS

One patient dropped out and the trial therefore totalled 24 patients Their ages ranged from 16 to 53 years Of these patients 23 completed the diary cards On breaking the code it was shown that 12 patients had received DSCG in the first period, and 12 had received placebo The groups were found to be comparable with respect to age distribution, sex, family history, duration of symptoms and the appearance of the nasal mucosa

The patients' evaluation of the treatment was favourable to DSCG in that 15 preferred DSCG, no patients preferred the placebo treatment and 9 had no preference (Table I)

Upon examining the nasal smears we found that no change in the presence of eosinophil had occurred during the trial period

The statistical evaluation of the diary card is shown in Table II Significant differences in favour of DSCG were found in all symptoms in the group of patients receiving placebo in the first period as well as for sneezing in the whole group There was no difference in the use of antihistamine tablets between the groups In Table III a specific analysis of patient cards from 14 patients with DSCG preference is shown A significant effect of DSCG in the group receiving placebo first for symptoms "running and sneezing" is also shown in Table III

Four patients showed positive intracutaneous tests when exposed to a number of foods and one of these also reacted to dogs' hair In 8 patients measurable concentrations of IgE in the nasal secretion were found and 15 showed increased IgE concentration in serum as compared with normal Five patients showed measurable IgM concentrations in the nasal secretion No correlation was found between these discoveries and the effect of DSCG treatment

The serum concentration of IgG was found to be significantly higher in 15 patients with preference for DSCG ($p<0.05$) than in the remaining 9 No other significant differences were shown in the other immunoglobulin fractions in serum (Table IV) or on the nasal secretion

No side effects were observed during the trial period

Table II *Wilcoxon's matched pairs signed ranks test of diary card monthly totals whole material consisting of 23 patients*

Variable	Drug order group	Mean monthly total for DSCG	Mean monthly total for Placebo	Wilcoxon T value	Significance
Blockage	DSCG–Placebo	20 5	18 3	35 (11)[a]	n s [b]
	Placebo–DSCG	28 5	35 8	9 (10)	$0\ 05<p<0\ 10$
	All patients	24 7	27 4	88 (21)	n s
Running	DSCG–Placebo	28 7	25 7	31 (10)	n s
	Placebo–DSCG	30 3	37 3	17 (12)	$0\ 05<p<0\ 10$
	All patients	29 5	31 7	96 (22)	n s
Sneezing	DSCG–Placebo	18 7	26 5	20 (11)	n s
	Placebo–DSCG	21 1	29 4	12 (11)	$0\ 05<p<0\ 10$
	All patients	20 0	28 0	59 (22)	$0\ 02<p<0\ 05$

[a] Figures in parentheses are the number of non tied pairs Tied pairs are excluded
[b] n s no significance

DISCUSSION

The diagnosis and treatment of perennial rhinitis has always been beset by considerable difficulties No strict delimitation between allergic rhinitis and vasomotor rhinitis has ever been defined In our material—as in a number of previous investigations—there has been no provable difference in the results of treatment between patients with a clearly allergic rhinitis and the others

The presence in nasal secretion of eosinophils in perennial rhinitis is of more limited diagnostic value than in hay fever because this test cannot be used to distinguish between allergic and vasomotor cases We did not find that treatment with DSCG changed the number of eosinophils This is not only in contrast to what has been the case in hay fever (Illum et al , 1973) but also in contrast to what other researchers have found regarding perennial rhinitis (Holopainen et al 1975, Girard & Bertrand, 1975)

Mygind et al (1972) found that an effect of DSCG may be expected in patients with nasal eosinophilia and a short duration of illness No correlation was found in the present study between the effect of treatment and the duration of illness

We found that the antihistamine consumption during the trial was an uncertain para

Table III *Wilcoxon's matched pairs signed ranks test of diary card monthly totals Selected material consisting of 14 patients with DSCG preference*

Variable	Drug order group	Mean monthly total for DSCG	Mean monthly total for Placebo	Wilcoxon T value	Significance
Blockage	DSCG–Placebo	20 6	17 9	19 (8)[a]	n s [b]
	Placebo–DSCG	25 0	36 2	1 (5)	n s
	All patients	22 5	25 7	34 (13)	n s
Running	DSCG–Placebo	31 1	28 6	19 (8)	n s
	Placebo–DSCG	27 8	43 8	0 (6)	$0\ 01<p<0\ 02$
	All patients	29 7	35 1	32 (14)	n s
Sneezing	DSCG–Placebo	22 5	34 8	7 (8)	n s
	Placebo–DSCG	21 3	39 7	0 (6)	$0\ 01<p<0\ 02$
	All patients	22 0	36 9	12 (14)	$p<0\ 01$

[a] Figures in parentheses are the number of non tied pairs Tied pairs are excluded
[b] n s no significance

Table IV *Immunoglobulins and albumin in serum in 15 patients with preference for DSCG and 9 patients without any preference*

Variable	With DSCG preference	Without DSCG preference	Significance
Albumin	45 33 g/l (0 74)	45 22 g/l (0.88)	n s
IgG	146 87 U/ml (8 37)	117 56 U/ml (6 32)	$p<0\ 05$ (Student s t)
IgA	108.53 U/ml (11 30)	93 67 U/ml (14 46)	n s
IgM	127 80 U/ml (10 98)	149 22 U/ml (26 36)	n s
IgE	140 U/ml	160 U/ml	n s

Figures in parentheses are standard deviations

meter Very few patients had a large consumption, as they had in the past, and the majority did not feel it necessary to supplement the spray treatment

It is remarkable that there was such a great difference between the groups 'DSCG first' and 'placebo first This agrees with findings of previous researchers and is probably due to the longer 'carry over' effect after the end of treatment than is normally taken for granted in DSCG treatment

Previously no attempts have been made to relate the effect of DSCG treatment in nasal complaints to the immunoglobulin concentration Many of our patients had increased IgE levels in serum but there seemed to be no correlation between this and the effect of treatment

This is in agreement with the fact that IgE concentration in serum reflects the degree of the patient s nasal complaint only to a minor degree due to the small size of the shock organ The fact that it has not been possible to prove a more distinct effect of DSCG in patients with clear presence of IgE in the nasal secretion should be noted

In our material it will be seen that we found significantly higher IgG serum concentration in patients preferring DSCG than in patients without preference However this immunoglobulin is only very rarely involved in allergic reactions type I and in these cases DSCG is found to be without any effect (Bryant et al 1973) No explanation of this finding can be given at the moment

The treatment of perennial rhinitis is often unrewarding Elimination of the disease causing allergens is often impossible Hyposensitization is a long and demanding procedure which can only be used when the specific allergen can be identified with reasonable certainty and even then the effect is often doubtful Antihistamine tablets often have a satisfactory effect on the nasal symptoms, but in many cases cause considerable side effects

We must therefore consider DSCG to be a valuable addition to the existing treatments for perennial rhinitis It is very atoxic and local side effects are insignificant and rare The new applicator with an exactly defined dose—without use of propellant gases—is a considerable improvement

ZUSAMMENFASSUNG

Doppelblinde Studien wurden an 24 Patienten mit chronischer Rhinitis durch Verwendung von 2% Disodium Cromoglycate (DSCG) Nasal Spray durchgefuhrt 15 Patienten zogen DSCG dem Placebo vor, der Rest wies keine Praferenz auf Bei der Auswertung von Patientenkarteien fand man hinsichtlich der Symptome Stenosis, Sekretion und Niesen einen signifikanten Unterschied zu gunsten von DSCG in der erst mit Placebo behandelten Gruppe ($p<0\ 1$) wahrend sich in der erst mit DSCG behandelten Gruppe kein Unterschied zwischen den Behandlungsperioden heraus stellte Bei den 15 Patienten mit Praferenz fur DSCG wurde signifikant hohere IgG Konzentration in dem Serum gefunden als bei den ubrigen ($p<0\ 05$) wahrend es ubrigens keine Anderungen in der Immunoglobulinkonzentration der Nasensekretion oder des Serums gab

die zur Wirkung des DSCG in Beziehung gesetzt werden konnten DSCG Nasal Spray wird als eine wertvolle Erganzung in der Behandlung von chronische Rhinitis betrachtet

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PENETRATION OF ERYTHROMYCIN STEARATE INTO MAXILLARY SINUS MUCOSA AND SECRETION IN CHRONIC MAXILLARY SINUSITIS

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Abstract The penetration of oral erythromycin stearate (Abboticin) administered in a dosage of 500 mg three times a day into the maxillary sinus mucosa and secretion was studied in 15 patients (22 sinuses) operated on for chronic maxillary sinusitis The average concentration in serum was 2 3 μg/ml 1 2 μg/ml in secretion and 1 8 μg/ml in mucosa These concentrations are highly effective against diplococci and most aerobic and anaerobic streptococci (MIC value 0 06 μg/ml) but not against *Haemophilus influenzae* (MIC value for 80% of 2 μg/ml)

The causative agents in acute paranasal sinus infections are mostly pneumococcus and haemophilus strains and in some cases β haemolytic streptococci and staphylococci (Axelsson & Brorson 1973 Eneroth & Lundberg 1976 Kalm et al 1975 Rantanen & Arvilommi 1973) In chronic sinusitis however *anaerobic* bacteria especially anaerobic streptococci are also more prominent The prerequisite for successful treatment is the use of an antibiotic with the right antimicrobial spectrum and adequate capacity to penetrate into the infection site

In maxillary sinus infections the concomitant increase in blood flow also increases the deposition of the antibiotic agent in the mucosa The amount of antibiotic which will subsequently reach the sinus lumen depends upon the extent of capillary leakage in the acute phase and in the chronic stage upon the secretory capacity of the mucosa Further more the amount of active antibiotic in the sinus secretion is dependent on the magnitude of the insoluble protein complexes formed

The concentration of various antibiotics in the sinus mucosa and/or secretion has been studied by several authors (Axelsson & Brorson, 1973, 1974, Eneroth & Lundberg 1976 Gnarpe & Lundberg 1971, Johnsen & Raunio 1975, Kalm et al 1975 Kohonen et al, 1975 Lundberg et al, 1968 1969, Lundberg & Malmborg 1971) Only a few of these studies deal with erythromycin however Axelsson & Brorson (1974) and Kalm et al (1975) studied erythromycin in serum and sinus secretion in acute cases whereas no data seem to be available on erythromycin concentrations in chronic maxillary sinusitis and on mucosal concentrations data are lacking for both acute and chronic stages New studies are needed because erythromycin has recently gained a more widespread use as new effective drugs against staphylococci have been developed and there is no longer any need to hold erythromycin in reserve

In the present study we have measured erythromycin concentration in the serum the maxillary sinus mucosa and the secretion of chronic maxillary sinusitis patients By chronic maxillary sinusitis we mean a disease which in radiological examination shows evidence of

marked mucosal hyperplasia, often polypous changes, and which does not heal despite repeated antral irrigations In histological examination such mucosa shows enormous thickening as compared with normal, inflammatory cell infiltration, increased fibrosis and frequently a marked increase in the secretory elements (Palva et al , 1962)

MATERIAL AND METHODS

The material consisted of 15 patients, in whom 22 maxillary sinuses were operated on for chronic maxillary sinusitis by the Caldwell Luc procedure The ages of the patients ranged from 16 to 65 years (mean age, 42)

All conservative methods for the treatment of maxillary sinusitis, such as repeated antral irrigations and antibiotics combined with anti histamines and nose drops, had failed in these patients All the sinuses contained purulent or mucopurulent secretion when last irrigated 2 weeks prior to surgery Detailed data on all the antibiotics that the patients had received from other physicians as treatment for the sinusitis were not available, but in most cases Penicillin V or tetracycline derivatives had been administered

The patients were premedicated with a com bination of atropine and pethidine or with a combination of morphine and scopolamine Six patients had their operation under general and 9 under local anaesthesia

The patients were given oral erythromycin stearate (Abboticin) in a dosage of 500 mg three times a day for 4 days before operation No other medication was given during 2 weeks before surgery The operation was performed 2½ h (±½ h) after the last dose of erythromycin

When there was secretion which was uncon taminated with blood a sample was aspirated through a cannula into a syringe from the opened maxillary sinus Simultaneously a sample of venous blood was drawn from the cubital vein, and a large piece of the thick sinus mucosa including the mucoperiosteum was obtained All three samples were sent to the laboratory for estimation of erythromycin concentration A second sample of mucosa was removed for histopathological examina tion

The concentration of erythromycin was determined with the punched holes technique according to Bennet et al (1966)

Samples for bacteriological analysis were taken from the nose before starting erythromycin therapy, from the secretion at operation, and from the returning fluid at irrigation if there was still some secretion at the post-operative visit

All the patients had a check-up 2–6 months after the operation and the operated sinuses were irrigated with saline

RESULTS

The bacteriological findings in 12 nasal swab specimens obtained preoparatively were pneumococci (2 patients), Haemophilus strains (2 patients) and β hemolytic streptococci (one patient) Staphylococci were identified in three and other bacteria also considered saprophytic, in four specimens One specimen yielded no growth The samples from the maxillary sinus secretion, taken after 4 days of erythromycin treatment, showed that 12 sinuses were sterile *Haemophilus influenzae* was cultured from three and *para influenzae* from two sinuses None showed diplococci β hemolytic streptococci were found in one sinus, anaerobic streptococci in one and staphylococcus in two sinuses Postoperatively all but three sinuses were free from secretion One had yellow staphylococci in culture and in one patient both sinuses showed continuous infection with *Haemophilus influenzae* Primarily the sinus system of this patient was extensively diseased with severely damaged mucosa in the nasal passages too and it has not been possible to bring the infection under complete control after surgery with any drug

An adequate sample of blood free secretion was obtained from 18 sinuses for the deter mination of erythromycin concentration The

Fraction III without adherent cells (Fraction III TLWOA)

Preparation of cultures

Tonsillar and blood lymphocytes were adjusted to a concentration of 10^6 cells/ml and 1 ml of each cell suspension was added to culture tubes (No 3001 Falcon plastics) The culture medium consisted of RPMI 1640 (Grand Island Biological Co) supplemented with 20% heat inactivated fetal calf serum Penicillin and streptomycin were added at 100 units/ml and 100 μg/ml respectively The cultures were incubated at 37°C in a humidified 5% CO_2 atmosphere In contrast to TLWOA and BLWOA TLWA and BLWA had a higher ratio of monocytes and granulocytes Therefore the lymphocytes were counted microscopically and the cell suspension was adjusted so that the lymphocyte concentration was 10^6 cells/ml of culture medium

FL cells (epithelial cell strain from human amniotic membrane Fogh & Lund 1957) were initially grown on 60×15 mm plastic culture dishes (Falcon) at a concentration of 10^6 cells per dish and fed with 5 ml of Eagle s Minimal Essential Medium (MEM) supplemented with 10% fetal calf serum 100 units/ of penicillin 100 μg/ml of streptomycin and glutamine

After 2 days of incubation at 37°C in a humidified 5% CO_2 atmosphere confluent monolayers of FL cells were formed and used for the assay of interferon

Lymphocyte stimulation

Stimulation by antigen (PPD allogenic cells) and by mitogen PHA was performed as described previously (Sugiyama et al 1976)

Assay of blastoid cell transformation found after lymphocyte stimulation

Incorporation of tritiated thymidine (^{3}H TdR) was assayed using the modification of a filter pad technique as described by Mans & Novelli (1961) A detailed method for assay has been described previously (Sugiyama et al 1976)

Interferon production by lymphocytes

(i) *Interferon induced by Newcastle Disease Virus (NDV)* For inducing interferon 1 ml of each lymphocyte culture was inoculated with 10^9 plaque forming units/ml of NDV which had been titrated on primary chick embryo fibroblast After 1 hr the inoculum was removed and replaced by the same volume of medium The supernatant fluids were harvested for interferon assay after an incubation period of 24 hrs They were centrifuged at 105 000 *g* for 2 hrs and dialysed overnight against citrate–HCl buffer at pH 2 Subsequent neutralization to pH 7 was achieved by adding NaOH solution These interferon preparations were stored at −20°C

(ii) *Interferon induced during the cellular immune response* After each lymphocyte culture received cellular immuno stimulation and PHA stimulation cell free supernatants were collected for assay of interferon at varying intervals These interferon preparations were stored at −20°C

Assay of interferon

Cell free supernatants were assayed for interferon by diluting the fluids in MEM supplemented with 5% fetal calf serum Appropriate dilutions were incubated for 18 hrs on duplicate FL-cell monolayer cultures After removal of the fluids the monolayers were washed twice with MEM and 100 Plaque Forming Units (PFU) of Vesicular Stomatitis Virus (VSV) were added to each cell culture Adsorption of the virus was allowed to proceed for 60 min at 37°C An overlay medium consisting of 1% Bacto agar (Difco) and MEM supplemented with 2% fetal calf serum 100 units/ml of penicillin 100 μg/ml of streptomycin and 1% glutamine was added to each cell culture After the cells were incubated for 48 hrs the cell cultures were stained with neutral red and the number of plaques was counted The interferon titer was considered to be the reciprocal of that dilution which produced a 50% reduction in PFU when compared with control cultures

Table I *Interferon production of each fraction induced by Newcastle disease virus (NDV) (each value represents the interferon titer per milliliter)*

	Ex 1	Ex 2	Ex 3	Ex 4	Ex 5
Fraction I[a]	195	100	80	250	N D
Fraction III[a]	1 120	140	150	300	N D
Fraction I[b]	95	60	N D	120	150
Fraction III[b]	N D	110	N D	390	450
Original Fraction	215	N D	120	N D	N D

[a] Tonsillar Lymphocytes With Adherent cells (TLWA)
[b] Tonsillar Lymphocytes Without Adherent cells (TLWOA)
Original Fraction Pre separated material prepared from tonsil for discontinuous density gradient centrifugation
N D Not done

Effect of interferon treatment on virus replication in tonsillar lymphocytes

Human interferon was produced by tonsillar lymphocytes in response to NDV according to the method described above Such interferon preparations were diluted to 20 units/ml by RPMI 1640 supplemented with 20% fetal calf serum Tonsillar lymphocytes separated from other subjects were passed through the cotton filled column described above The cells eluted from the column (TLWOA) were suspended at a final concentration of 10^6 cells/ml and were incubated for 18 hrs in the medium containing NDV induced interferon A control non interferon treated group was prepared in the same manner by incubating tonsillar lymphocytes from the same donor for 18 hrs in a medium not containing interferon The tonsillar lymphocytes were washed twice with RPMI 1640 and then resuspended at a concentration of 10^6 cells/ml Then 100 PFU of VSV were added At various intervals after infection, the supernatant fluids were harvested and their PFU content determined

Procedure to test whether the inhibitor can directly inactivate VSV

PFU were assayed on the monolayer FL cells after incubation of VSV for one hour at 37°C with inhibitor produced by lymph

response to PHA and with maintenance medium

Species specificity of interferon

The species specificity of interferon was determined by measuring the effect of interferon produced in human tonsillar lymphocytes on homologous (FL cells) and heterologous cells (mouse L cells, Kuchler & Merchant, 1956, and hamster BHK cells, Macpherson & Stocker, 1962) in the plaque-inhibition assay

Assay for T (thymus dependent lymphocytes) and B (thymus-independent lymphocytes) cells

The percentages of T cells, B cells, and peroxidase positive cells found in each fraction were determined as described previously (Sugiyama et al , 1976)

RESULTS

1 *The capacity of human tonsillar lymphocyte subpopulation separated by density gradient centrifugation to produce interferon*

We previously reported that lymphocytes isolated from human tonsil produced interferon in response to NDV infection of PHA stimulation (Sugiyama et al , 1972, 1974) In order to ascertain which subpopulation produces the most interferon, the original lymphocyte suspension from human tonsil (TLWA) was separated into three fractions by discontinuous density gradient centrifugation

Table II *Interferon production of each fraction induced by phytohemagglutinin stimulation (each value represents the interferon titer per milliliter)*

	Ex 1	Ex 2
Fraction I[a]	2.5	12
Fraction III[a]	8 5	22
Original Fraction	2 9	24

[a] TLWA
Original Fraction Pre separated material prepared from scontinuous density gradient centrifugation

Table III *Comparison between properties of Newcastle disease virus (NDV) induced and phytohemagglutinin (PHA) induced interferon in tonsillar lymphocytes*

Treatment	Effect on interferon induced by NDV	PHA
Centrifugation 105 000 g 2 hrs (supernatant assay)	−	−
56°C 1 hr	−	+
pH2 24 hrs	−	+
Trypsin (0 1 mg/ml 1 hr 37°C)	+	+
Dialysable	None	None

+ indicates a reduction in titer of inhibitor after treatment
− indicates no reduction in titer of inhibitor after treatment

Table I shows the results obtained when NDV was used as inducer The lymphocytes in Fraction III (the fraction with the highest specific gravity) seemed to produce more interferon than lymphocytes in Fraction I having a lower specific gravity However, there was a considerable difference in interferon titer from donor to donor

Table II shows the production of interferon by PHA stimulated tonsillar lymphocytes Again the lymphocytes in Fraction III produced the most interferon The material produced by lymphocytes in response to PHA stimulation was considered to be interferon because it inhibited the replication of VSV Since the lymphocytes were obtained from patients with recurrent tonsillitis the possibility existed however that an inhibitor acting directly on the virus was released To rule this out experiments were performed to determine if the substance released from lymphocytes in response to PHA had any direct inhibitory effect against VSV (see Materials and Methods) There was no difference in infectivity between interferon treated VSV and untreated VSV Therefore the inhibition of VSV replication was not by direct inactivation of the virus

A comparison was made between the characteristics of interferon induced by NDV and that of interferon induced by PHA It was found (Table III) that interferon induced by NDV was of the classical type (Type I) because it was stable to heat and low pH That induced by PHA was immune interferon (Type II) as it lost activity after exposure to low pH and heat Both interferons were species specific in that they were not active on mouse L cells and hamster BHK cells against the same challenge virus

Table IV indicates the ratio of T and B lymphocytes which constitute each of the three fractions differentiated by specific gravity Fraction III contained more B cells than the other fractions Since it also contained T cells it was impossible to determine whether T cells or B cells or both produced the interferon detected

2 *Sensitivity of human tonsillar lymphocytes to interferon*

The lymphocytes obtained from human tonsil were divided into two groups, one treated with 20 units/ml of human interferon and the other left untreated Both groups were infected with VSV and the infectivity of the supernatants determined at varying intervals As shown in

Table IV *Percentages of rosette forming cells in each fraction of human tonsillar lymphocytes separated by discontinuous density gradient centrifugation*

E Rosette formation with sheep erythrocytes (T cells)
EAC Rosette formation with EAC 3 (B cells)

	E (%)	EAC (%)	Peroxidase positive cells (%)
TLWA			
Fraction I	41 8	44 6	7 ?
Fraction II	46 7	42 4	5 8
Fraction III	25 8	72 1	2 2
TLWOA			
Fraction I	45 2	39 1	1 2
Fraction II	49 1	41 2	0 90
Fraction III	30 4	68 1	0 90

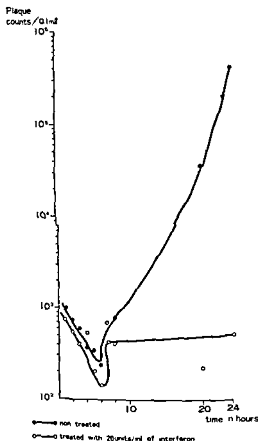


Fig 1 Inhibitory effect of interferon on VSV replication in tonsillar lymphocytes

Fig 1, VSV replication in lymphocytes treated with interferon was inhibited

Tonsillar lymphocytes in Fraction I (TLWOA) and III (TLWOA) were treated with 20 units/ml of human interferon and subsequently infected with VSV The results indicate (Table V) that lymphocytes from both fractions each having different specific gravity, were protected against VSV by interferon

3 *Blastoid transformation and interferon production of human tonsillar lymphocytes*

The authors previously reported the following (Sugiyama et al , 1976) Viable tonsillar and blood lymphocytes were separated from tuberculin sensitive tonsillectomized patients The degree of lymphocyte transformation of tonsillar lymphocytes without adherent cells (TLWOA) to PPD was minimal Blood lymphocytes responded far better to PPD than tonsillar lymphocytes did when adherent cells were absent However, tonsillar lymphocytes could react to allogeneic tonsillar lymphocytes in a mixed leukocyte culture reaction We have now studied lymphocyte production of interferon under similar circumstances and the results are presented below

Fig 2 shows the results of interferon production and blastoid transformation by PPD stimulation In the case of tonsillar lymphocytes from which adherent cells had been removed (TLWOA), the incorporation of ^{3}H TdR was extremely small and only a small amount of interferon was detected When the suspensions contained adherent cells (TLWA), both an increment of ^{3}H-TdR incorporation and production of interferon were observed The peak of interferon production tended to appear earlier than the peak of ^{3}H-TdR incorporation In the case of peripheral blood lymphocytes, they reacted to PPD even in the absence of adherent cells (BLWOA) and production of interferon was also observed

The results of similar experiments with mixed lymphocyte cultures are shown in Fig 3 Tonsillar lymphocytes reacted to allogeneic lymphocytes in that markedly increased incorporation of ^{3}H-TdR was observed over that in the control (unmixed culture) irrespective of the presence or absence of adherent cells Interferon production was observed in both TLWA and TLWOA cultures However the cultures containing adherent cells (TLWA) ex-

Table V *Inhibitory effect of interferon on VSV replication in tonsillar lymphocytes*

Each value represents virus titer (plaque forming units per milliliter) in medium at 24 hr after VSV infection

	Non treated	Treated by 20 units of interferon
TLWOA		
Fraction I	1.4×10^6/ml	2.3×10^4/ml
Fraction III	1.1×10^6/ml	2.1×10^4/ml

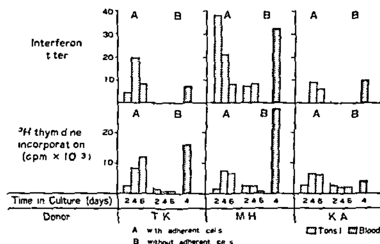


Fig 2 ^{3}H thymidine incorporation and interferon production by tonsillar and peripheral blood lymphocytes after stimulation with PPD

hibited higher incorporation of ^{3}H-TdR than the cultures without adherent cells (TLWOA) and somewhat more interferon was also produced

In the case of mixed lymphocyte cultures, the peak of interferon production appeared earlier than the peak of the incorporation of ^{3}H-TdR

DISCUSSION

It is known that tonsils produce humoral antibody to foreign antigenic substances and that tonsillar lymphocytes have the capacity to undergo cellular immunological responses (Surjan et al , 1969, 1970, 1971, 1971, 1972, Godrich & Patt, 1971, Tada & Ishizaka, 1970, Enomoto et al , 1974, Tabata et al , 1974, Watanabe et al , 1974) This indicates that tonsillar lymphocytes play an important role in the immunological defense against virus infections The present study was conducted to determine whether or not tonsillar lymphocytes produce interferon a substance important in host defense It could be shown that tonsillar lymphocytes produce classical interferon in response to NDV and that they produce immune interferon as a part of the cellular proliferative response to mitogen and antigen just as has been observed for peripheral blood lymphocytes Korsantiia et al (1974) cultivated fragments of resected tonsils and exposed the supernatants of such cultures to pH 2 and found an interferon like substance in abo 50% of the specimens He reported that in fluenza virus or para influenza virus could b isolated from the tonsils which were produc ing interferon These authors concluded tha the tonsil produces interferon in response t virus infections

Our study revealed that the lymphocytes o Fraction III having higher specific gravity produced more interferon than lymphocyte from the less dense fractions Kinoshita et al (1970, 1974) reported that the Fraction III i composed of lymphocytes having superio immunological activity Wallen et al (1973

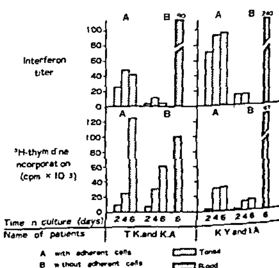


Fig 3 ^{3}H thymidine incorporation and interferon production by tonsillar and peripheral blood lymphocytes after stimulation in mixed leukocyte cultures

took the spleen cells from mice, separated them by albumin discontinuous density gradient centrifugation into six fractions, stimulated the cells of each fraction by various mitogens and PPD, and investigated mitogenesis and interferon production in each fraction The cells which produced interferon appeared mostly in the fraction (which they called Fraction V) having the 5th heaviest specific gravity of all the fractions This fraction is different from the fraction in which most of the cells which proliferate in response to mitogen are found Wallen et al also suggested that the interferon producing cells do not belong to the thymus-dependent lymphocyte subpopulation and that at least two cell types were required for the production of interferon in response to PHA or PPD stimulation Epstein and coworkers (1974) separated human blood lymphocytes into T cells and B cells by using a fluorescence activated cell sorter and found that both human T and B cells can respond to PHA and PWM *in vitro* in the presence of macrophages with proliferation and production of interferon They also reported that T cell interferon production and proliferative responses can be assessed at 3 days in culture while B cell interferon and their proliferative response is delayed to 5 and 7 days When these findings are taken into account, it may be estimated that interferon produced by mitogen is produced both by T and B cells and that the Fraction III obtained by us must have contained the Fraction V obtained by Wallen et al Our Fraction III contained relatively large amounts of B cells but it also contained T cells Since it is the fraction in our system in which most of the immunologically active cells are found, we expected that this fraction would produce the most interferon and it did

When NDV was used as inducer the lymphocytes from Fraction III also produced the most interferon It is interesting to note that virus induced interferon production is highest in the fraction which had the greatest proliferative response It is assumed that lymphocytes of Fraction III have a greater capacity to produce interferon per cell than do the cells of other fractions Glasgow (1966) reported that when peritoneal leukocytes from mice immunized with Chickengunya virus (CV) were harvested and the CV-immune and non immune leukocytes were cultured and infected by CV, the CV immune leukocytes produced from two to ten times as much interferon as a similar number of non immune cells At this time it is not clear whether an immune recall phenomenon similar to that described by Glasgow is operative in the human leukocyte–NDV system as an explanation for the high interferon titers observed when cells from Fraction III were challenged with NDV

When VSV was added to tonsillar lymphocytes in vitro, proliferation of the virus was observed Such infection was suppressed in the case of tonsillar lymphocytes treated by human interferon as compared with untreated lymphocytes This suggests that interferon produced by tonsillar lymphocytes is protective against infection by viruses There was no difference between Fraction I and Fraction III in regard to the sensitivity of tonsillar lymphocytes to the protective effects of interferon

Human tonsil lymphocytes produced interferon in response to PPD and in the mixed lymphocyte culture reaction The peak of such interferon production tended to appear earlier than the peak of ^{3}H-TdR incorporation an indicator of the extent of blastoid transformation of lymphocytes It is of interest that Gifford et al (1971) reported that in the mixed culture of peripheral blood lymphocytes from mice, the peak of interferon production likewise appeared earlier than the peak of ^{3}H TdR incorporation

ZUSAMMENFASSUNG

Tonsillare Lymphozyten produzierten in Reaktion auf die NDV Infektion klassisches Interferon (Type I) und in Reaktion auf PHA PPD oder histo-incompatible Antigen immunes Interferon (Type II) Die tonsillaren Lymphozyten die höheres spezifisches Gewicht haben

und die starkere proliferative Reaktion gegen Mitogen oder Antigen zeigen, produzierten mehr Interferon als diejenigen Lymphozyten, die nederes spezifisches Gewicht haben Dieses Phanomen wurde auch beim durch Virus infizierten Interferon beobachtet In der Empfindlichkeit gegen den schützenden Effekt von Interferon gab es keinen Unterschied zwischen den schweren und den leichten, kleinen tonsillaren Lymphozyten In bezug auf das Interferon, das im Zusammenhang mit der zellularen immunen Reaktion produziert wird, zeigte sich die Neigung, daß der Hohepunkt der Produktion von Interferon eher als der Hohepunkt der Inkorporation von ^{3}H-TdR erscheint Diesen Ergebnissen kann entnommen werden, daß die tonsillaren Lymphozyten in der Abwehr des Menschen gegen die Infektion durch Virus eine wichtige Rolle spielen

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— It should be noted that ampicillin, like phenoxymethylpenicillin, does not penetrate into sinus secretions from blood in reliable concentrations. —

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MACULA UTRICULI IN FOUR CASES WITH MENIERE'S DISEASE

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Abstract Four patients suffering from Meniere s disease were labyrinthectomized Maculae utriculi were removed and studied by electron microscopy The neuro-epithelia from all four patients appeared fairly normal considering the age of the patients However, two types of degenerative change which could be related to the inner ear disorder could be distinguished One was vacuolation of the sensory cell cytoplasm followed by pycnosis of the nucleus and cell death The other was cystic degeneration This started with localized separations between the sensory cell and the nerve chalice These separations developed into a single cystic cavity A few large intra-epithelial cysts were found probably representing the final stage of the cystic degenerative process

Meniere's disease is still an enigma, and very little is known about the pathogenesis of the syndrome Severe clinical symptoms are not yet correlated to distinct and typical morphological changes Recent research indicates that Meniere's disease, at least in its early stage, could be a metabolic disturbance of the inner ear, apparently without loss of hair cells (Schmidt et al , 1974) In light microscopic studies a distension of the endolymphatic space, first described by Hallpike & Cairns in 1938 is invariably found, yet the vestibular sensory epithelia often appear normal (Schuknecht et al , 1962) However, a number of authors have found ultrastructural changes affecting both sensory and supporting cells and which could possibly be attributed to Meniere s disease Such changes are loss of sensory hairs, extensive vacuolation of sensory cell cytoplasm, and accumulation of fat droplets (Pietrantoni & Iurato, 1960, Litton & Lawrence, 1961, Ireland & Farkashidy, 1963, Friedmann et al , 1963, Hilding & House 1964, Sanchez Fernandez & Marco, 1975, Colman et al , 1975) However, as many of these authors have emphasised, it is difficult to establish if these changes can be attributed to the disease or if they could depend on other factors, such as aging and preparation artifacts

MATERIAL AND METHODS

The material consisted of specimens from 4 patients who suffered from severe, unilateral Meniere s disease Relevant clinical data concerning the patients are found in Table I In each patient the affected ear had shown progressive, sensorineural hearing loss and Bekesy, ABLB and stapedius reflex tests had indicated cochlear lesion All patients had experienced severe and frequent attacks of disabling vertigo a condition which necessitated labyrinthectomy, since medical treatment had failed Transtympanic labyrinthectomy was performed in cases 1, 2 and 4, and transmastoid labyrinthectomy in case 3 The postoperative course was uneventful in all 4 cases

In all cases the utricle was removed during the operation and immediately immersed in a fixative 1 5% veronal buffered osmic tetrox

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*1 77*947

Table I *Clinical data of the four Meniere cases shortly before operation*

Case no	Age at time of operation	Duration of symptoms (years)	ENG findings: Spontaneous or positional nystagmus	ENG findings: Caloric responses	Audiological findings: Pure tone average (500–2 000 Hz) (dB)	Audiological findings: Glycerol test
1	34	7	None	Normal	75	Negative
2	40	8	None	Strongly reduced	70	Negative
3	59	3	None	Strongly reduced	75	Positive
4	60	10	None	Reduced	65	Positive

ide was used for this purpose in cases 1, 3 and 4 In case 2 the specimen was first fixed in a 2 5% phosphate-buffered glutaraldehyde, then rinsed with water and post-fixed with osmic tetroxide The specimens were dehydrated in alcohol and propylen-oxide They were embedded in Epon 812 and cut on an LKB Ultrotome Ultrathin sections were mounted on copper grids and stained with uranyl acetate and lead citrate The ultrastructural studies were done with a Siemens Elmiscope 1A

RESULTS

In all 4 cases a general observation indicated that the macula utriculi had a fairly normal toarchitecture Type I and type II sensory lls could be identified The type I cells were urrounded by nerve chalices (Fig 1C) Synaptic regions with synaptic bodies could be identified at the base of the sensory cells (Fig 1B) In the younger individuals (cases 1 and 2) the surface structures appeared normal, with hair bundles protruding from the sensory cells and numerous microvilli from the supporting cells (Fig 1A) In case 3 the surface of the sensory epithelium was denuded of cilia, but microvilli could still be found (Fig 2A) In case 4 only scattered remnants of hair bundles could be identified

The cytoplasm of the supranuclear part of both sensory and supporting cells contained large amounts of lipofuscin inclusions (Fig 2A, B) In the sensory cells these inclusions were composed of an electron dense matrix surrounded by a membrane In the supporting cells the inclusions consisted of round, osmiophilic vesicles surrounded by granular masses (Fig 2B) These inclusions were very numerous in the macula utriculi from the older individuals (cases 3 and 4) In case 2, the 40-year old patient, they were found, but they were neither so large nor as common as in the older cases In case 1, the 34 year-old patient, lipofuscin inclusions were very rare

Occasional laminated inclusions were observed in the infracuticular region of many sensory cells from cases 2, 3 and 4 (Fig 2A) Also, *striated inclusions* were sporadically seen in the extracellular space close to the basement membrane

Among the majority of sensory cells which appeared normal considering the age of the individual, there were many cells showing signs of degeneration Two distinct types of degeneration could be discerned One was a granulation and vacuolation of the sensory and supporting cell cytoplasm Initially, the cytoplasm appeared dark, filled with osmiophilic granules (Fig 3A) Later, it contained numerous vacuoles and degenerated mitochondria (Fig 3B, C, D) The border between the nerve chalice and the type I sensory cell was wavy (Fig 3D)

In some cells the vacuolation was excessive, with large vacuoles filling up the entire cytoplasm (Fig 4) The nerve chalice, which had shrunk considerably, contained an electron dense granular material and numerous mitochondria (Fig 4) The final stage of this degenerative process was the destruction of the sensory cell



Fig 1 (A) Surface area from the macula utriculi case 1 In the centre of the photomicrograph the apical part of a normal looking type I sensory cell is seen A hair bundle is protruding from the sensory cell and microvilli from the surrounding supporting cells ×17390 (B) Synaptic bar (*arrow*) at the basal part of a type I cell Case 1 ×26320 (C) Normal looking type I cell surrounded by a nerve chalice Case 2 ×14100

The other type of degeneration affecting only *type I* cells, was a separation between the sensory cell and the nerve chalice The first sign was the formation of diminutive cavities Later these cavities merged and expanded forming one single cystic space (Fig 5A B) The sensory cell, dislocated by the cystic cavity, appeared shrunk Its cytoplasm

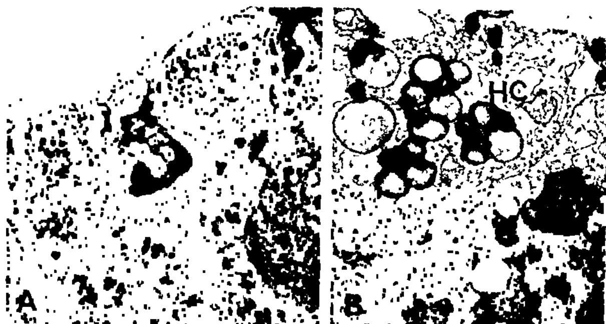


Fig 2 (A) Apical part of sensory epithelium with numerous lipofuscin inclusions Case 3 ×8500 (B) Lipofuscin inclusions in a hair cell (*H C*) and in a supporting cell (*s c*) Case 4 ×15000

was filled with dark osmiophilic granules and large vacuoles, and the nucleus was pycnotic (Fig. 5A)

A few very large cysts were found in case 2 (Fig 6A) These cysts were located entirely within the sensory epithelium, and were surrounded by dislocated sensory and supporting cells. The cells in the vicinity of the cysts frequently exhibited severe vacuolation (Fig. 6A) The walls of the cyst consisted of a single layer containing cytoplasmatic organelles and fibrillar structures (Fig 6B) The origin of this cyst lining could not be established A small number of microvilli protruded from the wall into the cystic space (Fig 6B)

In Table II a summary of the ultrastructural finding is given

DISCUSSION

In spite of the severe auditory and vestibular dysfunction in the 4 cases described here, macula utriculi in all 4 cases exhibited only minor ultrastructural alterations However, both sensory cells and supporting cells were filled with osmiophilic lipofuscin inclusions, abundantly seen in the 2 older patients. Vestibular sensory epithelia from normal individuals also contain such inclusions (Hilding & House, 1964; Ishii et al., 1967). A comparison between normal vestibular neuroepithelia (Rosenhall & Rubin, 1975), and those described here, reveals that the lipofuscin inclusions occur as abundantly in the normal sensory epithelium as they do in that of a Meniere-stricken inner ear. Also, the laminated inclusions seen in the apical part of the sensory cells, as well as the striated bodies (long-spacing collagen) observed extracellularly in the basal part of the neuroepithelium, have been observed in normal subjects (Hilding & House, 1964; Friedmann, 1967; Rosenhall, 1974*a*, Rosenhall & Engström, 1974) Accordingly, the presence of all inclusions observed in our study, cannot be related specifically to Meniere's disease. However, they can be explained as part of a normal aging process.

Profound surface alterations, such as loss of cilia, have been attributed to Meniere's disease by several authors (Pietrantoni & Iurato 1960, Ireland & Farkashidy, 1963; Friedmann

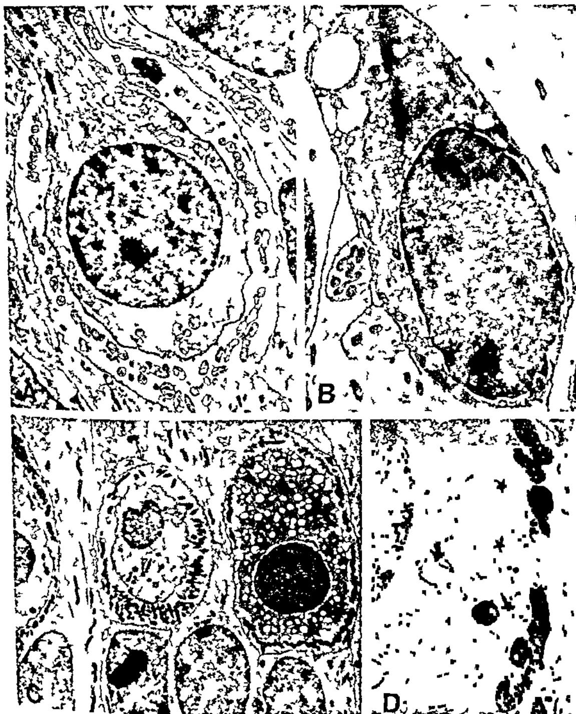


Fig 3 (A) Type I cell from case 1 The sensory cell cytoplasm is granulated ×9500 (B) Granula on and va cuola ion of the cytoplasm from a cell n the macula u ricu of ca e ? ×10500 (C) Th ee type I cells two normal looking and one w h ex en ve vacuola on and granulat on (*a row*) Case 2 ×6000 (D) The same degenerated sensory cell as shown n (C) Degenera ed m tochondria are seen (*arrow*) The border between the sensory cell and the nerve chal ce s a y (s ars) ×19500

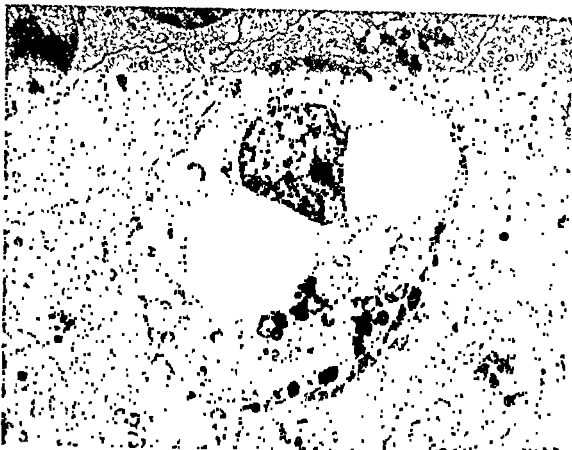


Fig 4 Severe vacuolation of the cytoplasm and pycnosis of the nucleus in a type I cell Case 3 ×10 500

al , 1963, Harada, 1973, Sánchez-Fernández & Marco, 1975) In the present study the sensory cells from the younger individuals had normal-looking hair bundles One of the two older cases had some fairly normal hair bundles, while the other was denuded of cilia

Loss of sensory hairs in older individuals has been regarded as a preparation artifact, perhaps reflecting an increased brittleness of the cilia (Hilding & House, 1964; Rosenhall & Rubin, 1975). Accordingly, the lack of cilia in the older individuals in this study probably does not reflect inner ear disease

A prominent finding in Meniere's disease is vacuolation of vestibular sensory cell cytoplasm (Pietrantoni & Iurato, 1960, Friedmann et al , 1963; Hilding & House, 1964, Sánchez-Fernández & Marco, 1975) The presence of

Table II *Ultrastructural changes found in macula utriculi of the four Meniere cases*

Case no	Lipofuscin inclusions	Surface alterations	Vacuolation or/and granulation	Cystic separations	Intra epithelial cysts	Illustrations Fig No
1 (34 years)			+	–	–	1A 1B 3A
2 (40 years)	(+)		+ +	+ +	+	1C 3B 3C 3D 5A 5B 6A 6B
3 (59 years)	+ +	+	+ +	(+)	–	2A, 4
4 (60 years)	+ +	+ +	+ +	+ +	–	2B



Fig. 5. (A) Cystic separation (stars) between nerve chalice and degenerated type I cell. Case 2. ×10 500. (B) The nerve chalice lining the cystic cavity seen in (A). ×32 000

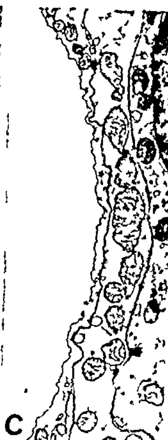
this type of degeneration is confirmed in this investigation. In normal individuals of corresponding age a similar granulation of sensory cells can also be observed, but to a much lesser extent.

Vacuolation of sensory cells in the vestibular apparatus has also been observed in experimental animals after administration of ototoxic drugs (Wersäll & Hawkins, 1962; Kanda & Igarashi, 1969). Obviously, this kind of degeneration is an unspecific indication of an ongoing destructive process.

A more specific pattern of degeneration was also observed in the present material. This degeneration starts with a separation between the type I sensory cells and their nerve chalices. The sensory cells undergo vacuolation and pyknosis in a way similar to the vacuole degeneration already described. The separation leads to the formation of a large cystic space, which probably interferes severely with the neural function.

Intra-epithelial cysts, similar to the cystic cavities seen in Menière cases, have been observed in normal vestibular sensory epithelia in experimental animals (Werner, 1933; Lindeman, 1969). Watanuki & Kageyama (1973) and Engström et al. (1974) have shown that these cysts develop from nerve structures.

The large intra-epithelial cysts, occasionally seen in the present material in one of the cases, might represent the final stage of the cystic separations between the sensory cells and their nerve chalices. However, a morphological difference exists between the two kinds of cyst. The inner lining of the large intra-epithelial cysts contains a few microvilli, not seen in the cystic separations. In addition,


C


B



microvilli are never found in those intra epithelial cysts which are seen in normal animal inner ears (Fig 6 C)

Large cysts, often multilocular, are frequently seen in the vertical cristae from elderly individuals (Rosenhall, 1974*b*) These cysts have numerous microvilli attached to the cuboidal cells, which constitute the inner lining of each cyst (Fig 6 D)

It is possible that all these different cystic changes seen in vestibular sensory epithelia from normal experimental animals, from aged humans and from Meniere patients, may have a common background They might represent different expressions of a basically similar degenerative process, provoked by very disparate agents, and affecting sensory cells and nerve chalices

ZUSAMMENFASSUNG

Vier Patienten mit Menieres Krankheit sind mit Labyrinthektomie operiert worden Die Macula utriculi wurden dabei entfernt und mit Elektronenmikroskopie studiert Wenn man das Alter der Patienten beachtet hatten die Sinnesepithele aller vier Patienten ein ziemlich normales Aussehen Es wurde festgestellt daß es zwei verschiedene Arten von degenerativen Veränderungen gibt die man auf die Innerohrkrankheit zurückführen kann Eine Art von Degeneration war Vacuolenausfüllung der Haarzellenzytoplasma worauf Pyknose und Zellentod folgten Die andere Art war eine zystische Degeneration Diese fing mit der Trennung der Haarzellen von dem Nervenkelch an und entwickelte sich später zu einem einzigen großen zystischen Lochraum Einige große intraepithelielle Zysten wurden auch wahrgenommen und diese vertreten wahrscheinlich das Endstadium des zystischen degenerativen Prozesses

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Fig 6 Comparison between different types of intra-epithelial cyst (A) Large intra-epithelial cyst from case 2 ×4 900 (B) Close-up picture of the wall lining the cyst shown in (A) Microvilli are protruding into the lumen of the cyst ×15 500 (C) The wall of an intra-epithelial cyst from a squirrel monkey No microvilli are seen ×18 000 (D) The wall of a cyst in the posterior crista from a 75-year-old woman Numerous microvilli are protruding into the lumen of the cyst ×15 000

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ACTION OF ALCOHOL ON VESTIBULAR COMPENSATION AND HABITUATION IN THE CAT

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Abstract Several effects of alcohol on vestibular nystagmus are well known including positional alcoholic nystagmus a depressive effect on post rotatory nystagmus and an inhibition of visual fixation This study concerns the influence of alcohol on central vestibular compensatory phenomena In one experiment hemilabyrinthectomized cats were allowed to compensate for the postoperative spontaneous nystagmus and directional preponderance of post rotatory nystagmus Following alcohol injection spontaneous nystagmus reappeared toward the intact side positional nystagmus was unidirectional and post rotatory nystagmus was profoundly more depressed than for normals Normal cats subjected to repeated accelerations showed less habituation of post rotatory nystagmus with alcohol than without

Alcohol influence on vestibular function in man has been studied for many years Aside from considerations of alcohol effects on posture and gait, and on the possible etiology of alcoholism there have been numerous investigations of the direct effect of alcohol in causing nystagmus with the head tilted from its normal upright position (Goldberg 1966) Positional alcohol nystagmus (PAN) has recently been explained on the basis of strictly mechanical considerations in the peripheral end organ (Money & Myles 1974, Money et al 1974) In this paper, we are not concerned directly with the question of PAN, but rather with the possible interactions between the drug and central modification of vestibular responses as measured through the vestibulo-ocular reflex It has been well established that the 'vestibulo-ocular reflex' is subject to variation by a variety of means Orientation information from visual and other sensory modalities (Melvill Jones & Gonshor, 1975), habituation, arousal and instructions (Collins, 1975) have all been shown to modify vestibular nystagmus Furthermore, a number of pharmacological agents are known to interfere with the normal vestibular responses In particular alcohol depresses nystagmic reaction to angular acceleration of man in the dark, but enhances it in the light due to its suppressive effect on the visual fixation mechanism (Schroeder, 1971) Our purpose in the present experiments was to investigate the effects of *alcohol on compensation and habituation* manifested in vestibular nystagmus

Compensation of the asymmetrical postural reflexes and eye movements following unilateral vestibular lesions has been shown to be modifiable by pharmacological agents Schaeffer & Meyer (1973) reviewed the consequences of the administration of stimulating drugs, which they have shown to accelerate compensation They assume that the progress is mediated, at least in part, by non specific generalized arousal On the other hand, depressant drugs such as barbiturates slow the

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process of compensation of eye and head reflexes, even in doses which have little direct effect on vestibular responses Under some circumstances, the effects of unilateral lesions, already compensated, may reappear with administration of a depressant drug (Russel, 1894–1895) Schaeffer & Meyer (1975) suggest the same hypothesis which motivated the present experiments, that alcohol may exert a comparable influence and abolish compensation to vestibular lesions

Habituation refers specifically to response decline upon repeated stimulation with identical rotational stimuli Collins (1975) reviews the evidence that central mechanisms are responsible for habituation to repeated acceleration in animals The experimental literature concerning effects of pharmacological agents on habituation is ambiguous, although some evidence exists to indicate that drugs which maintain a high level of arousal (such as amphetamines) reduce the habituation effect By contrast, in the cat, drugs which maintain arousal or other alerting means, are apparently ineffective in eliminating the habituation of feline vestibular nystagmus on repeated acceleration Aschan (1967) demonstrated the loss acquired vestibular habituation in pilots n administration of alcohol However, no ysiological studies of the effect of alcohol on these central influences has been undertaken

Our hypothesis was that the centrally determined habituation or compensation of the vestibulo-ocular reflex would be suppressed upon administration of alcohol and that the vestibulo-ocular reflex tested after alcohol administration would consequently indicate, to some extent the uncompensated or non habituated response To test the effect of alcohol on compensation hemilabyrinthectomized (HL) cats were subjected to vestibular rotation and positioning stimulation at various times following the operation For the investigation of habituation normal cats were subjected to repetitive angular acceleration in the dark with and without alcohol

METHODS

Eleven adult cats were used in the study, of which 2 were reserved for controls and 2 were used in the preliminary pilot experiments All cats showed normal bidirectional horizontal vestibulo-ocular reflexes before the operation In all but 2 control cats, a right hemilabyrinthectomy was performed The cats were anesthetized with ether, the bulla was approached ventrally and the inner ear was visualized after removal of the first turn of the cochlea The horizontal canal and utricular nerve were sectioned and the labyrinth destroyed by suction and lesion of the bony structures containing the other vestibular nerves Cats were allowed at least 4 days to recover before any tests were applied A period of 8 days to one month elapsed between surgery and the alcohol injection In 2 cats alcohol injections were made, one week and one month respectively after surgery, in order to test the compensation at different stages All vestibular rotation tests were performed with the cat held rigidly in a normal head orientation (placing the plane of the lateral semicircular canal approximately horizontal) They were restrained by a tightly wrapped binding around the trunk and legs The head was immobilized using a fast-setting plaster and restrained with metal pins connecting the head to the stereotaxic frame, permitting no observable motion For tests of positional nystagmus, the entire frame was rolled 90 degrees in each direction about a horizontal axis, to place the right or left ear down Horizontal eye movements were measured using electro oculography with Grass subdermal electrodes placed in the skin at the outer canthi and with the neutral electrode above the nose Vertical eye movements were monitored in some experiments using a pair of electrodes placed above and below one eye

For rotation stimuli, the cats were placed on a velocity servo turntable which rotated the animals about a vertical axis passing approximately between the two labyrinths The

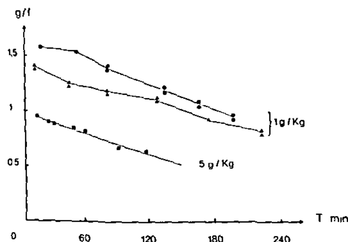


Fig. 1. Time course of blood alcohol. Time course *T* blood alcohol plotted in grams per litre (g/l) for 3 different cats which received 5 g/kg and 1 g/kg of 95% alcohol intravenously (see text).

standard velocity profile was a succession of 30 second duration, ±60 degree/second velocity steps (with a rise time of less than 200 msec). The animal was blindfolded during the vestibulo-ocular reflex tests, which were performed prior to alcohol injection, and every 20 to 30 minutes thereafter. After each rotation test, the animal was checked for spontaneous nystagmus and positional nystagmus with left and right ear down. Optokinetic tests were performed by rotating the animal in each direction in the light, with a view of the normal laboratory surround for periods up to 120 seconds at a constant velocity of 60 deg/sec. To reduce variability in nystagmus, we attempted to maintain arousal level by appropriate auditory stimulation.

Eye motion recordings were calibrated by equating the ocular slow phase angular velocity, measured during periods of optokinetic nystagmus steady state tracking, with the speed of the turntable rotation, 60 deg/sec. (This method produces a consistent, although unknown, overestimation of eye movements.)

Alcohol was injected intravenously in the femoral vein. Dosages were calculated to lie in the range of 0.2 to 2 gms/kgm and were diluted to 5 to 10% solution in Ringer's solution. The alcohol was perfused steadily over a ten minute period. To monitor the actual blood alcohol level, blood samples were taken periodically following the injection in each experiment. An enzymatic method of alcohol analysis was used (Joffrey and Serveaux, 1943). This method requires only very small samples of blood (0.1 ml per sample). The alcohol sample was treated with hemolyne, centrifugated, and then incubated in the presence of an alcohol dehydrogenase. The precision of the method and its specificity leads us to prefer it to the chemical method of oxidation reduction.

For the experiment on habituation, 2 intact cats were subjected to 23 repeated trials of horizontal rotation with angular velocity square wave tests in the dark. Four days after the control test, the same animals were subjected to the identical rotation paradigm following a 10 minute injection of 1.5 g/kg alcohol. The alcohol injection was preceded by a single control rotation to verify that the vestibular nystagmus strength had returned to its original level.

DATA ANALYSIS

For each vestibular test, the nystagmus resulting from the first full (120 deg/sec) step pair to the left and to the right was utilized. The EOG records were visually analysed to determine the peak frequency of fast phase of nystagmus, averaged over the peak 5 sec period of the response (15 sec for the habituation study). The duration of the nystagmus

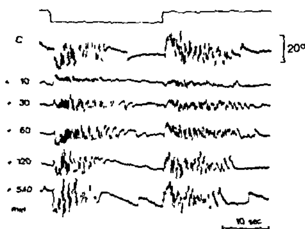


Fig 2 Post rotatory nystagmus in the dark of a normal cat before and after alcohol injection Top line turntable velocity Successively two steps of 120 deg/sec Clockwise (or right side) rotation is up C is control before alcohol Successive tests for +10, +30, +60, +120, and +540 min after alcohol injection Approximate calibration was obtained from optokinetic slow phase velocity Eye movements to the right are shown by upwards deflections Note the longer duration, smaller amplitude of post rotatory nystagmus after alcohol

was measured from the step of table angular velocity until the last recorded fast phase in the primary post-rotatory nystagmus direction. Additionally, the cumulative eye position (CEP) was calculated for the 10 or 15 sec following the step in table angular velocity.

P represents the sum of the amplitudes of ..ch of the fast phases, and is an approximation to the cumulative slow phase eye deviation during the epoch

RESULTS

Blood alcohol levels

The blood alcohol level appeared to decrease linearly with time at a rate from 0 16 to 0 18 gram/liter/hour Typical levels for three cats are shown in Fig 1

Control experiment Normal cats injected with alcohol and with pure Ringer s injection

Two cats with intact labyrinths were subjected to the same alcohol dosage and optokinetic and vestibular stimulation paradigms as were the HL cats. Typical vestibular nystagmus responses to left and right velocity steps are shown for one such animal in Fig. 2 C represents the control rotation prior to alcohol injection, whereas the other traces indicate vestibular nystagmus to the identical rotation stimuli at the indicated times following onset of alcohol injection.

Note the roughly symmetric vestibulo-ocular reflex response to left and right rotations. The response was markedly reduced, both in amplitude and frequency, but not in duration, for the first test pair at 10 min post

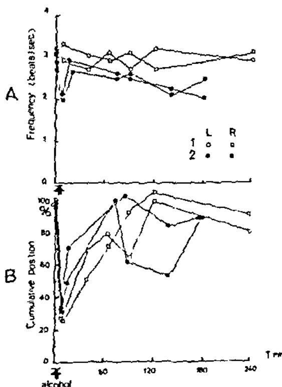


Fig 3 Culmination frequency and cumulative eye position of post rotatory nystagmus in the dark for normal cats (A) Mean frequency of post rotatory nystagmus (PRVN) during the peak 5 sec following the step of velocity (cf Fig 2) The initial value is the control just prior to alcohol injection *T* indicates time Data from 2 cats (1 and 2) For each cat the velocity step was to the left (*L*) or counterclockwise and to the right (*R*) or clockwise Note the small decrease of PRVN frequency (*B*) Cumulative eye position during the peak 5 sec following the step ... as in (A) Cumulative eye ... ntrol value ... ation of in- ... A and B ... the decrease after 120 min may be ... is habituation acting in the opposite direction to recuperation

alcohol injection, and recovered over the following trials to approximately the control value at 120 minutes Beat frequency and 10 sec cumulative position for the 2 control cats is given in Fig 3 The initial depression of nystagmus strength and its recovery is especially marked in the plot of cumulative position Positional alcohol nystagmus corresponding to PAN I (fast phase towards the ear that is down) developed following elevation of blood alcohol, and faded as the blood alcohol level fell A rotatory component was also observed

To ensure that the depressive effects on nystagmus were attributable to alcohol and not to the restraint, habituation, or the injection, a control test was performed using an intact cat injected with a standard volume of Ringer's solution containing no alcohol Except for a brief depression of nystagmus frequency and cumulative eye position lasting less than 5 min after the injection, there was no effect of the Ringer's solution injection on subsequent vestibular tests The depression of vestibular nystagmus as seen in intact cats following alcohol injection was, therefore, attributable to the alcohol and not the restraint or the fluid injection

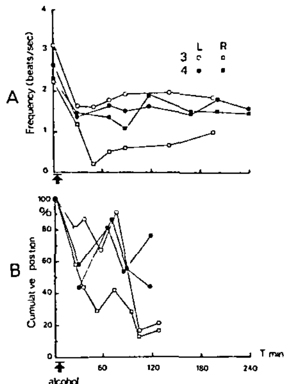


Fig 5 Frequency and cumulative eye position for post rotatory nystagmus in the dark for hemilabyrinthectomised cats (lesion on right side) Same notations as in Fig 3 for two HL cats (3 and 4) Note the continued depression of both frequency and cumulative position over the whole range of experiment duration

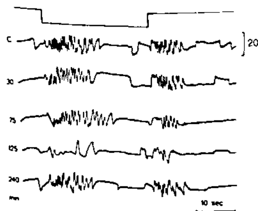


Fig 4 Post rotatory nystagmus in the dark of a hemilabyrinthectomised cat (lesion on right side) Post rotatory nystagmus after velocity step under the same condition as for Fig 2 C is control just prior to alcohol injection Successive tests for +30 +75 +240 min after injection Note continued depression of PRVN up to 125 min

Compensation of cats to hemilabyrinthectomy

Each HL cat presented the classical signs of ataxia (Magnus, 1924), falling toward the lesioned side, spontaneous nystagmus with fast phase towards the intact side, and a lack of vestibulo-ocular reflex on acceleration toward the operated side Compensation was observed to develop over a period of weeks and months following the operation The initial spontaneous nystagmus and absence of response to acceleration toward the operated side was gradually replaced by a more nearly balanced vestibulo-ocular reflex upon acceleration in each direction

Effects of alcohol on HL cats during rotation

Since neither the appropriate levels of the blood alcohol nor the precise time course of

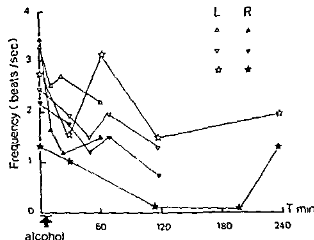


Fig 6 Frequency of post rotatory nystagmus in the dark of hemilabyrinthectomised cats Same notation as in Fig 5 This figure gives additional data on 3 cats for rotations to the left (unfilled symbols) and to the right (filled symbols)

the development of compensation were known prior to this experimental series, a range of alcohol dosages were utilized and animals were tested at various times following hemilabyrinthectomy A typical set of vestibulo ocular reflexes to rotation in the dark, for one such animal, is indicated in Fig 4 The pre-injection control test (C) indicates only a slight directional preponderance towards the left (intact) side Following alcohol injection (1 g/kg), nystagmus strength is reduced for rotations in both directions, just as in the case of the normal cats shown in Fig 2 The response of HL animals however, remains reduced for a considerably longer time following alcohol than is the case for the intact cats The duration of vestibular nystagmus for rotations to the left becomes increasingly larger than that for rotations to the right The time course of the depression of the vestibulo-ocular reflex was quite varied among the different animals On occasion, we observed a complete absence of vestibular nystagmus following a rotation step, but only a movement of the eye in the anti compensatory direction which remained displaced for several seconds and fell back to the midline position, following a time course reported by others (Melvill Jones 1964)

Four cats were carried through the complete HL compensation period and subjected to alcohol injection and rotation Two of the cats were given repeated tests at different periods following the operation The peak nystagmus frequency and cumulative eye position measured over the maximum 5 sec of activity for rotations to the left (intact) and right (operated) sides are indicated in Fig 5 Frequency alone is shown for one retest, and for the 3 other cats in Fig 6 For the operated cats, the vestibular nystagmus peak frequency was more depressed in both directions than for the intact control cats (Fig 3) and remained depressed for a longer period of time None of the HL cats tested returned to its pre alcohol level during the maximum test period of 240 min Following injection of alcohol, the depression of nystagmus strength was somewhat greater for rotations to the right (the initially impaired direction) than to the left among the

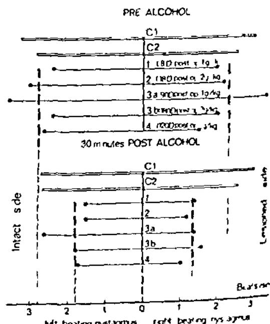


Fig 7 Summary of experiments for normal and hemilabyrinthectomised cats for pre alcohol and 30 min post alcohol injection Mean post rotatory nystagmus frequency measured as in Fig 2 and Fig 4 before and 30 min after alcohol injection in 2 control cats (C1 and C2) and 4 hemilabyrinthectomised cats Time after operation is indicated for each cat as well as dose of alcohol [illegible] operation) All cats [illegible] alcohol [illegible]

Table I *Peak nystagmus beat frequencies for HL cats, pre-alcohol and 30 minutes post alcohol*

Cat number	Frequency pre alcohol			Frequency 30 minutes post alcohol					
	Left	Right	Average	Left	(% pre)	Right	(% pre)	Average	(% pre)
4-3	2.4	2.0	2.2	1.5	(63)	1.4	(70)	1.45	(66)
7-2	3.0	2.2	2.6	1.5	(50)	1.2	(55)	1.35	(52)
8-2	3.5	3.5	3.5	2.6	(74)	1.4	(40)	2.0	(57)
8-3	2.4	2.0	2.2	1.8	(75)	1.6	(80)	1.7	(77)
9-2	2.6	1.5	2.0	1.7	(65)	1.0	(67)	1.35	(66)
Average	2.78	2.24	2.51	1.82	(65)	1.32	(62)	1.57	(64)

Pairwise analysis
Pre alcohol versus Post alcohol (left and right)
Pre alcohol (2.51) Post alcohol (1.57)
$t = 5.04$ 4 d f Δ means=0.94
($p < 0.01$)
Pre alcohol (left–right)
$t = 2.86$ 4 d f Δ means=0.54
($p < 0.05$)
Post alcohol (left–right)
$t = 2.47$ 4 d f Δ means=0.50
($p < 0.10$)
Post alcohol percentage of pre alcohol (left–right)
$t = 0.37$ 4 d f Non significant

HL cats, whereas no such directional effect was seen for the intact animals.

Peak nystagmus frequency prior to alcohol injection, and for the trial closest to 30 min after the beginning of this injection, is shown for the HL animals in Table I, and graphically along with data from the controls in Fig 7. The 2 control cats had balanced left–right nystagmus peak frequencies prior to alcohol injection. These figures had not changed noticeably by 30 min after the initiation of the dosage. In contrast, even prior to alcohol injection, each of the HL animals showed a somewhat larger peak nystagmus frequency for rotation to the left (intact) rather than to the right (operated) side. By 30 min following initiation of the alcohol injection, the HL animals showed a significant decrease in nystagmus peak frequency for rotations in both directions. Furthermore, the response to rotation to the right remained significantly lower than that for rotations to the left, 30 min after alcohol injection. The effect of alcohol on the left–right difference, however, was not significant.

For the HL cats, the most striking measure of the prolonged depressive effects of the alcohol injection was in the measure of cumulative eye position. Plots of CEP for two HL cats at various trial times following alcohol injection are shown in Fig 5. For neither cat was any significant recovery seen during the 2 hours of the test. Whereas cat 3 showed a much greater depressive effect for rotations to the right than to the left, little directional difference was seen for cat 4.

Positional nystagmus

The ocular response to positioning of the HL cats with right or left ear down was highly variable. One measure of the extent of compensation to the hemilabyrinthectomy was the observation that no residual spontaneous nystagmus was present for the upright position prior to injection of alcohol, and that only a negligible to moderate left beating nystagmus was evident in either the right ear or left ear down orientation. Following alcohol injection, however, 3 of the 4 animals showed a return of spontaneous left beating nystagmus. For at least 2 of the animals, the peak in the occurrence of this spontaneous nystagmus was at some time less than 30 min following alcohol injection, corresponding to a period when the depressive affect of alcohol on the vestibulo-ocular reflex frequency was marked. As seen in Fig 8, the effect of orienting the animal with either right ear or left ear down was to trigger a substantial level of left beating nystagmus following alcohol injection. In the experimental results illustrated for cat C, the

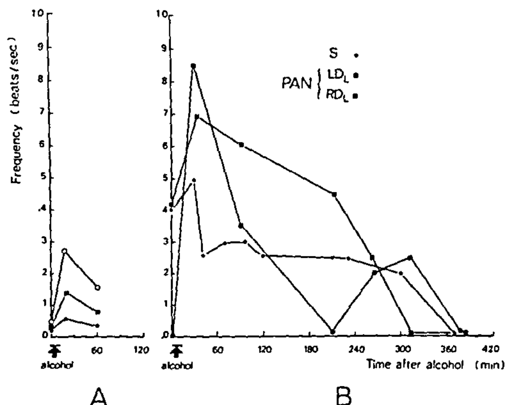


Fig 8 Examples of spontaneous and positional nystagmus frequency in the dark in 2 hemilabyrinthectomised cats In both cats (A and B) injection of alcohol released some weak spontaneous nystagmus (S) which was left beating (same direction as after lesion) Positional alcohol nystagmus (PAN) was tested with head left side down (LD) and right side down (RD) Direction of PAN was always left (indicated by the small letter L in LD_L and RD_L) Note the increase of positional nystagmus within 30 min after injection and the long duration of the effect in cat B

, cay of the left beating positional nystagmus ver the 6 hours of this experiment roughly parallels the decrease in blood alcohol level In all cases position nystagmus observed following alcohol injection was left-beating, as was any spontaneous nystagmus observed in the post alcohol period

Habituation experiments

Both intact cats showed essentially the same response to repeated rotation in the habituation series Both with and without alcohol there was a substantial depression of the strength of vestibular nystagmus in repeated trials. The nature of the habituation response with alcohol was somewhat different however For the alcohol test, the duration of the post rotatory nystagmus on the fifteenth trial exceeded that for the control test and the general nature of the nystagmus with alcohol was more of a continuing low amplitude wave form The frequency of nystagmus during the peak response period, and the cumulative eye position during this period, are plotted in Fig 9 The decrease in beat frequency for post alcohol trials paralleled that of the control habituation sequence for the first 5 to 10 trials With continued repetition of the stimulus however, the alcohol trials indicated no further reduction in the nystagmus beat frequency The same overall depression in nystagmus strength with alcohol found in the compensation experiments, was again revealed with intact cats In Fig 9*b*, a similar effect is shown in cumulative eye position In the control test, this cat showed a rapid

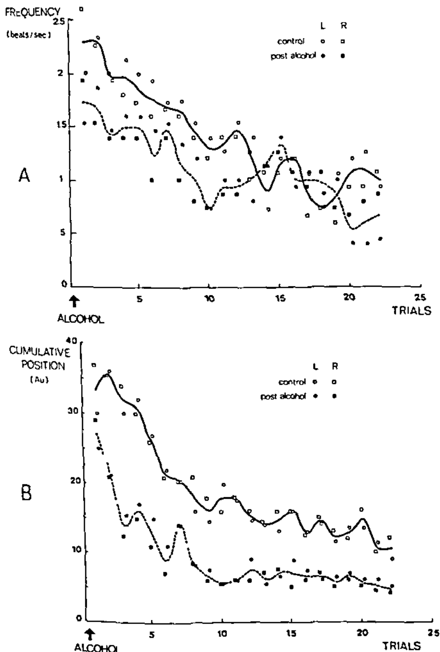


Fig 9 Effect of alcohol on habituation in a normal cat Post rotatory nystagmus was elicited in the dark in the same conditions as for previous figures Beat frequency (*A*) and cumulative position (*B*) are expressed as a function of repetition (trials) of the steps of velocity to the left (*L*) and to the right (*R*) Control values (unfilled figures) and mean curves (dark continuous line) are compared with post alcohol values (filled symbols) and corresponding mean curve (dotted line) See text for experimental conditions

decline in CEP over the first 10 trials, and a continuing diminution of cumulative eye position during the entire sequence (Jeannerod et al, 1975) For the alcohol test, on the other hand, this cat displayed no further reduction in CEP beyond the tenth trial A reduction in nystagmus duration over the first five trials was noted, both for the control and for the al

cohol senes Following the fifth tnal the dura tion of nystagmus for alcohol tests consistent ly exceeded that for the same animal in the control tests and at times was exceedingly long

DISCUSSION

The depressive effect of alcohol on vestibular nystagmus in normal cats rotated in the dark is consistent with Bárány s (1911) conclu sions and confirms the findings of Schroeder (1971) in man The depression is seen in both frequency and CEP measures but nystagmus recovers quickly Other groups have observed similar suppression of vestibular nystagmus in *man following alcohol ingestion (Ey 1964* Bochenek & Ormerod 1962 Mizoi et al 1965) When dealing with cats which have par tially or fully compensated from a hemilaby rinthectomy however the alcohol effect is a more profound and longer lasting suppression of vestibular nystagmus We are led to the possibility that the alcohol effect is on the compensation mechanism itself Russel (1894–95) observed the reappearance of compen sated ocular deviation in dogs (resulting origi nally from a cerebral cortex lesion) when ether s administered at the surgical anesthesia evel Schroeder (1971) demonstrated an effect of alcohol as interfering with the ability of subjects to suppress vestibular nystagmus by using visual fixation In our experiments we see another example of the interference of alcohol with a central modification of vestibular responses Of course a complete removal of compensation would have been evidenced by the virtual elimination of nystagmus upon rotation toward the operated (right) side In fact no significant difference in the depressive effect of alcohol was observed between vestib*ular responses in the two directions However* the appearance of spontaneous nystagmus toward the intact side following alcohol injec tion and the covariation of this nystagmus with blood alcohol level supports the conten tion that alcohol interfered with the compensa tory mechanisms and released the spontane ous nystagmus seen post operatively (Aschan et al 1964) The existence of position nys tagmus which always beats towards the intact side regardless of which ear was down lends further weight to the effect of alcohol in releas ing the non compensated responses although this finding is complicated by the direct (PAN I) effect of alcohol on the intact cupula (Money et al 1974 Nito et al 1968 Ooster veld 1973)

The second central mechanism wh ch we expected to be suppressed by alcohol was habituation to repeated vestibular stimuli among normal cats Aschan (1967) showed that the vestibular response of pilots normally *reduced during periods of active flying* was raised toward non habituated levels following alcohol ingestion Collins et al (1973) also speculated on the possibility that alcohol can enhance vestibular responses by releasing a central habituating mechanism Our results on effects of alcohol on the habituation to re peated acceleration indicate that although the habituation process was far from eliminated by the alcohol dose employed the habituation was neither as marked nor as prolonged as without alcohol

Both the HL compensation results and the habituation experiments support the sugges tion that alcohol has a depressive effect on vestibular nystagmus in darkness and that it suppresses the development and maintenance of centrally generated modification of this response

ACKNOWLEDGEMENTS

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ZUSAMMENFASSUNG

Mehrere Einflusse des Alkohols auf den vest bularen Nystagmus s nd bekannt z B der alkohol sche Pos tionsnystagmus e ne Verm nderung des postrotatorischen Nystagmus und e ne Hemmung der v suellen F xation D eser

Nach Kompensation des sich ergebenden spontanen Nystagmus und der direktionalen Preponderanz des postrotatorischen Nystagmus wurde Alkohol eingespritzt Infolge dessen erschien ein spontaner Nystagmus gerichtet gegen die intakte Seite der Positionsnystagmus war einseitig und der postrotatorische Nystagmus wurde bedeutend mehr unterdruckt als bei normalen Katzen Wurden normale Katzen wiederholten Beschleunigungen ausgesetzt zeigten sie unter Einfluß des Alkohols weniger Gewohnheitssymptome als im normalen Zustand

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LABYRINTHINE INPUT TO THE VESTIBULAR NUCLEI OF THE AWAKE CAT

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Abstract The labyrinthine input to the vestibular nuclei was investigated in 24 awake cats Stimulus consisted of electrical shocks given through bipolar silver wire electrodes implanted in the utricular and lateral ampullar nerves Throughout the vestibular nuclei single units were recorded extracellularly with glass micropipettes filled with Fast Green The tracts of the penetrating electrodes were identified histologically In all four nuclei units responding to both labyrinths outnumbered unilaterally responding neurones with certain differences between the individual nuclei Excitatory as well as inhibitory responses were observed polysynaptic being more common than mono- or disynaptic ones No monosynaptic contralateral responses were seen The latency distribution of contralateral responses closely mirrored that of ipsilateral responses within each nucleus Both excitatory and inhibitory responses fell into relatively segregated populations based upon latency distribution This implies separate pathways for labyrinthine input to the vestibular nuclei

Early studies (e g Cajal 1909) demonstrated that most, if not all, primary vestibular fibres dichotomize into an ascending and descending branch when they enter the vestibular nuclear complex (VNC) The ascending fibres supply the superior vestibular nucleus (SVN) and superior part of the lateral vestibular nucleus (LVN) while the descending branches give off collaterals ending in the descending vestibular nucleus (DVN) and the medial vestibular nucleus (MVN) (Hauglie-Hanssen, 1968) There is not evidence of primary fibres ending in the contralateral VNC (Walberg et al , 1958)

Within each of the main nuclei there are extensive regions free from vestibular afferents (Walberg et al , 1958) It follows from the anatomical distribution of primary vestibular fibres that impulses from the labyrinth cannot activate all parts of the VNC monosynaptically

As noted by Peterson (1970) all four vestibular nuclei contained cells driven monosynaptically or polysynaptically by labyrinth stimulation However, Peterson used decerebellate, anaesthetized cats Kimm & Luschei (1971) have shown that neural activity in the VNC varies with alterations in the physiological state of the animal and Yegorov (1962) found a considerable decrease in amplitudes of individual VNC potentials in decerebellate animals Possible anatomical substrates for multisynaptic excitation of VNC units may be either internuncial neurons in the VNC or collaterals of the axons leaving the VNC (Brodal et al , 1962, Lorente de Nó, 1933)

De Vito et al (1956) have demonstrated that neurons located in the LVN can be influenced by polarizing currents applied to the contralateral labyrinth, a finding that has been confirmed by Weber & Steiner (1965), Fredrick

son et al (1966), Shimazu & Precht (1966) Kasahara & Uschino (1971)

The present study was designed to investigate and compare the response latencies to labyrinthine stimulation within the four main vestibular nuclei

METHODS

Experiments were performed on 24 adult cats (body wt 2–3 5 kg) operated on under a halothane/N_2O/O_2 anaesthesia Tracheotomy was employed and entidal pCO_2 was monitored continuously with a Beckman gas analyser and kept at 3 5 to 4 5% Blood pressure was monitored and maintained above 80 mmHg by intravenous infusion of Ringer solution and occasionally Metarminol Bitartrate (Aramine) A heating pad placed under the animal maintained a constant body temperature (36°–37°C)

To avoid the depressive effect, the anaesthesia was discontinued prior to recording The animal was immobilized with gallamine triethiodide (Flaxedil) and positive pressure ventilation begun Strict precautions were taken to ensure that the animal experienced no pain Wounds and pressure points were repeatedly infiltrated with a local anaesthetic (Xylocaine HCl 2%) No signs of piloerection and complete miosis throughout the experiment testify to the absence of pain

The bulla was exposed via a ventro lateral approach rendering the middle ear accessible The vestibule was enlarged, exposing the nerve branches The stimulating electrodes (bipolar Teflon coated silver wires 125 μm insulated diameter) were secured in place on the utricular and the ampullar nerve branches, bilaterally

A major factor to be considered in the electrode implantation was stimulus spread to other nerves Thus in 6 of the cats bipolar chlorided silver electrodes were affixed to the anterior branch of the vestibular nerve, the cochlear nerve within the modiolus and the facial nerve at the genu Current spread was controlled by threshold comparison for these electrode pairs, observing evoked eye deviation and neuronal activity It was found that to record evoked potentials in the VNC, one needed to stimulate the cochlea with at least four times the intensity required for the vestibular nerve (T), and for the facial nerve at least nine times Thus using values of less than 3 5 T for vestibular nerve stimulation current spread was avoided

Glass microelectrodes (1–3 MΩ) filled with 2 M KCl, saturated with fast green FCT (Thomas & Wilson 1965) were employed At various recording sites, dye marks were deposited electrophoretically to aid in the histological localization of electrode tracks

The electrodes penetrated the intact cerebellum at a 30° angle, prior to reaching the VNC The presence of the electrode in the VNC was determined by stereotaxic aiming coordinates and the physiological technique of Shimazu & Precht (1966) The electrodes recorded extracellular activity and signals were amplified at a band width of 0 001–3 kHz in order to allow display of field potentials and neuronal activity on the oscilloscope for photography Low frequency was cut off at 300 Hz for triggering standard pulses by spikes to be displayed in post stimulus time histograms (PSTH) Unit activity and the monitored stimulus were also stored on analogue tape for off line analysis (Sigma 5 computer) The real-time relationship between stimulus and response was preserved

Mechanical stability of recording arrangements was achieved by covering the exposed area with 3% agar gel and when necessary by performing a bilateral pneumothorax Once the VNC was reached, the microelectrode was very slowly lowered while stimulating the ipsilateral labyrinth at 1 Hz, until a unit was encountered Nerve stimuli consisted of square waves (0 1 msec duration) with a frequency of 0 4–1 Hz Using ipsilateral stimulation the response latencies were recorded Variable bin widths were employed to detect early as well as late excitatory and/or inhibitory re

Table I *788 analysed neurons displayed in four groups due to their location in the main vestibular nuclei superior medial descending and lateral nucleus (SVN MVN, DVN LVN)*
The responses to ipsilateral contralateral or bilateral labyrinth input are indicated

Units receiving input from	SVN	MVN	DVN	LVN	Total
Ipsilat labyrinth	22 (34%)	125 (40%)	88 (41%)	75 (38%)	310
Contralat labyrinth	12 (19%)	10 (3%)	30 (14%)	14 (7%)	66
B lat labyrinth	30 (47%)	177 (57%)	97 (45%)	108 (55%)	412
Total	64 (100%)	312 (100%)	215 (100%)	197 (100%)	Σ-788

sponses A similar procedure was then employed for the contralateral labyrinth The spontaneous activity was also recorded

To classify the units the monosynaptic latency grouping of Wilson et al (1967) was employed All units that responded within the latency range of 0 8–1 5 msec were classified as monosynaptic Units with a response latency of 1 6–2 2 msec were classified as disynaptic (Ito et al , 1969) All other units were classified as polysynaptic

The animals were killed by a lethal injection of Pentobarbital (60 mg/kg), and a subsequent perfusion with 10% formalin solution Serial 50 μm frozen sections in the plane of the electrode tracks were stained with Klüver Barerra chnique and the dye marks located The oundaries used to separate the individual vestibular nuclei were those described by Berman (1968) According to his nomenclature, the regions explored with the recording electrode correspond to the superior medial lateral and descending nuclei

RESULTS

Discharge activity

788 VNC units were found to respond to electrical stimulation of the ipsilateral and/or contralateral labyrinth The average spontaneous discharge of vestibular neurons was 19±7 impulses per second Action potentials ranged from 30 to 150 μV, while resting potentials averaged 69 3±3 2 μV Stimulation of the vestibular nerve was also found to inhibit some neurons in the ipsilateral VNC 86% of the ipsilateral labyrinth responsive un showed an inhibitory response This inhibito response usually followed an excitatory r sponse but occasionally preceded it and w for 14% of the inhibited units the only r sponse seen

The majority of units in this study (40% n=788) were found to be located in the MVN Approximately the same percentage of uni was found in the LVN and DVN (25% ar 27%, respectively) Fewest units (8%) we recorded in the SVN This reflects to som extent differences in number of electrod tracks in the nuclei

Laterality of responses

The largest number of VNC units (52%, n 788) responded to bilateral labyrinth stimula tion Ipsilateral responses were more commo than contralateral ones (39% versus 8%) I Table I the four different VNC nuclei are com pared with regard to side of labyrinthine input In all four nuclei the largest number of unit responded to bilateral labyrinth stimulus wit the percentage ranging from 57% in the MVN to 45% in the DVN The next largest grou of units for all four nuclei were those respond ing to the ipsilateral labyrinth ranging from 41% in the DVN to 34% in the SVN Th smallest group included units activated from the contralateral labyrinth

Excitatory response latency distribution

Both excitatory and inhibitory responses were observed upon stimulation of the labyrinth Fig 1 shows the excitatory response latencies

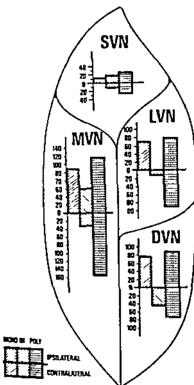


Fig 1 For each vestibular nucleus excitatory response latencies have been divided into three groups mono-synaptic (*mono*) d synaptic (*di*) and polysynaptic (*poly*) The number of responses are plotted against the three latency populations Ipsilateral responses are indicated above the X-axis and contralateral below

within the four individual nuclei The latencies have been divided into three groups mono-synaptic, disynaptic, and polysynaptic activation

In all nuclei, polysynaptic responses to ipsilateral or contralateral labyrinth input outnumbered monosynaptic or disynaptic responses

In the SVN (Table II) there were nearly as many disynaptic units as there were polysynaptic ones (37% versus 40% units) with ipsilateral stimulation In fact the SVN contained the largest percentage of disynaptic units in the VNC In response to contralateral labyrinth stimulus the number of disynaptic units was smaller No units responding monosynaptically to contralateral labyrinth stimulation were found in the SVN or any other vestibular nucleus

In the DVN relatively few disynaptic responses were noted (12%) as compared with mono or polysynaptic units using ipsilateral stimulus The number of monosynaptic units in the DVN (42%) was about the same as the number of polysynaptic units (46%) The number of disynaptic units responding to contralateral labyrinth activation was increased relative to the number of polysynaptic units

The largest number of monosynaptic responses was observed in the MVN However, still more polysynaptic responses were noted (Table II) Although the number of disynaptic responses in the MVN was greater than elsewhere in the VNC the relative percentage of such responses was less than that in the SVN or LVN For contralateral stimulation the vast majority of responses were of polysynaptic nature

In the LVN the conditions were similar to those in the DVN The number of polysynaptic units was close to the number of monosynaptic units For contralateral stimulation most of the responses were of polysynaptic nature and even fewer disynaptic units were noted than was the case when using ipsilateral labyrinthine stimulus

The largest overall percentage of units in the VNC (45%) was of polysynaptic types, followed by monosynaptic (37%) and disynaptic (18%) unitary discharges

Fig 2*a* illustrates the post stimulus interval histogram (PSIH) for excitatory responses to

Table II *The neurons in the four main vestibular nuclei are divided into different synaptic groups based on their response latencies 1 5 msec mono(synaptic), 1 6–2 2 msec di(synaptic) and 2 3 msec poly(synaptic)*

	SVN	MVN	DVN	LVN
Mono	23%	36%	42%	38%
Di	37%	19%	12%	18%
Poly	40%	45%	46%	44%
	100%	100%	100%	100%
	(*N*=64)	(*N*=312)	(*N*=215)	(*N*=197)

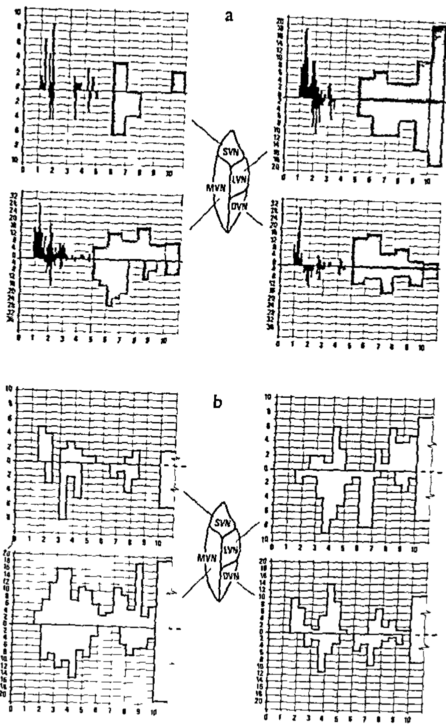


Fig 2 For each nucleu composite post stimulu time histogram of excita tory responses is displa ipsilateral above the X a (time in msec) and contr lateral below, number of neurons on the Y-axis T excitatory responses are shown in (*a*) The bin width during 0–5 msec is msec and during 5–10 ms 0 5 or 1 0 msec The inhibitory responses are shown in (*b*) the bin wid being 0 5 msec

ipsilateral and contralateral labyrinthine stimulation Certain features are apparent The responses fall into relatively discrete populations based upon their latency distribution

In the SVN, five separate groups appear One population includes only monosynaptic responses, while a second one is comprised of disynaptic responses only. Three more grou with latencies less than 10 msec can be o served

Contralateral responses mirror closely t latency distribution seen for ipsilateral r sponses The major difference is the absen of a monosynaptic group

The MVN and LVN do not show such a discrete grouping of response latencies as seen in the SVN or the DVN (Fig 2a) Nevertheless mono- and disynaptic groups appear to be present in both nuclei although one group blends into the other Other latency groups of less than 10 msec are not as distinct although the LVN MVN and DVN all appear to contain a large group of responses ranging from 5 1 to 10 msec Earlier latency groups are either not present or too difficult to discriminate

As seen in the SVN contralateral response groups tend to mirror closely the ipsilateral groups throughout the VNC The latency ranges of ipsilateral versus corresponding contralateral groups are very similar

Inhibitory response latency distribution

Fig 2b shows the PSIH for inhibitory responses in the VNC to bilateral labyrinth stimulation Due to the difficulty of determining onset of inhibition larger bin widths were employed than for excitatory responses Inhibitory responses also tend to fall into groups based upon their latency range Furthermore contralateral groups behaved like the ipsilateral ones with respect to number of groups present and their latency ranges

The SVN contained three response latency groups of less than 10 msec when ipsilateral vestibular nerve stimulus was used The earliest group had latencies of 1 6 to 2 msec which indicates disynaptic inhibitory pathways from the labyrinth to the VNC Such early inhibitory responses were seen throughout the VNC except for the LVN where the earliest ipsilateral responses were in the 2 6 to 3 msec range

The DVN also appeared to contain the three latency groups seen in the SVN In the MVN the boundaries of these groups tended to merge and distinct latency groups were impossible to determine The LVN contained only two distinct groups

The earliest contralateral response recorded was at least 0 5 msec later than was the ipsilateral one In the LVN however the onset of contralateral responses was more rapid and seen 1 msec *prior* to the earliest ipsilateral neuronal discharge

In the SVN MVN and DVN the contra and ipsilateral groups are quite similar as regards latency ranges and number of populations In the LVN there appear to be three groups of responses to contralateral stimulation whereas there are only two groups answering to ipsilateral stimulus In addition the latency ranges do not mirror each other to the extent seen in the rest of the VNC

DISCUSSION

The majority of units in the VNC received bilateral labyrinth input and were often reciprocally active i e when stimulus applied to one labyrinth evoked an excitation then stimulation of the opposite labyrinth resulted in inhibition of neuronal discharge However due to the nature of the stimulus a great deal of the reciprocity may be masked Thus units were occasionally found which could be inhibited or excited from both sides Whether this has a functional significance or is merely an artefact due to the stimulation technique is not clear However Desole & Pallestrini (1969) investigating the LVN of guinea pigs employing caloric stimulation observed excitation from either side This is in disagreement with the results of other investigators who found inhibition instead of activation following galvanic stimulation of the contralateral labyrinth (DeVito et al 1956) or predominance of units of type I (Duensing & Schaefer 1958) On the other hand Shimazu & Precht (1966) and Markham (1968) who studied the effects of electrical stimulation of the vestibular nerve on units of type I and type II found that only units of type I were inhibited while those of type II were excited There is little doubt that the commissural inhibitory system is brought into play to mediate the switching of activated sets of inhibitory and excitatory neurons in

one VNC to that in the other (Precht, 1974) The active influence from the ipsilateral and contralateral labyrinth will assure the highly sensitive responses of vestibular neurons and provide a basis for precisely organized efferent effects such as those on the oculo motor apparatus under both normal and pathological conditions (Baker & Berthoz, 1974) Further more, it has been postulated that the central processing of synaptic input at the vestibular membrane may play an important role in the response adaptation of the entire system to a motion stimulus (Kirsten, 1975)

As found by Peterson (1970) all four major vestibular nuclei contained cells driven mono- or polysynaptically by a labyrinth stimulus He found DVN to be the nucleus with the largest proportion of monosynaptic connections However, the percentage in his study (53%) was greater than that in ours (42%) A possible cause of this difference is that he used decerebellate, anaesthetized cats As the output from the cerebellar cortex is solely in hibitory, this may have resulted in fewer excitatory units being available for recording Kimm & Luschei (1971) have shown that neural activity in the VNC varies with alterations the physiological state of the preparation, d Yegorov (1962) found a considerable decrease in amplitudes of induced VNC potentials in decerebellate animals

The nucleus with the smallest percentage of monosynaptic cells was the SVN (27%) in this study, whereas it was the MVN (15%) in Peterson's Our investigation unlike his, made a distinction between di and polysynaptic units It cannot be ruled out that some of the very early disynaptic units in the SVN were in fact monosynaptic However it is equally possible that some of Peterson's late monosynaptic units in the SVN were actually disynaptic Evidence of the existence of these disynaptic units was strengthened by the work of Ito et al (1969) Recording from the LVN, they found that 29% of their units responded mono synaptically to vestibular stimulation This should be compared with 43% in Peterson's study, 39% in the study of Shimazu & Precht (1966) and 37% in the present study As the recording procedures and condition of the animals were different in the various studies differences are to be expected

As seen in Table II, disynaptic units were recorded in all vestibular nuclei This confirm the results of Kawai et al (1969) Thus th speculation by Ito et al (1969) about excita tory and inhibitory interneurons located i other vestibular nuclei, receiving powerfu monosynaptic, activation from primary ves tibular fibres, is not necessarily true

In the MVN, Peterson (1970) found onl 15% of the units to respond monosynaptically whereas Shimazu & Precht (1966) found 34% and the present study showed 36% Fredrick son et al (1966) in their study found 52% o the units in the area of the MVN–DVN borde responding monosynaptically Although ou results agree with those obtained by Shimazu & Precht, the differences mentioned agai stress the effects of different experimenta conditions

In addition to the VNC cells driven mono and disynaptically many were observed with polysynaptic connections The largest per centage of the later category was located in the DVN (46%) while the LVN appeared to contain the smallest part (39%) Peterson (1970) found the highest percentage of poly synaptic neurons (disynaptic ones included) in the SVN (40%) and the smallest (10%) in the ventral portion of the LVN Wilson et al (1968) had a corresponding figure of 11% fo the LVN whereas in the Shimazu & Precht study one can estimate that in the LVN, 51-61% of their cells were of polysynaptic nature

Sans et al (1972) found two groups of re sponses in the VNC upon electrical stimula tion of the VIII nerve One group, said to be type I, had a very short latency (1 0 msec) whereas the second group, type II showed a longer latency (1 3 msec) No mention was made of other response latencies Employing the criteria by Wilson et al (1966) both these responses are monosynaptic As they looked

only at neurons devoid of spontaneous activity, the cell sample is selected

The speculation that response latency distribution can be divided into discrete populations seems to be supported by the present study. Distinct latency groups may imply distinct pathways for the labyrinth input entering the vestibular nucleus in question. Thus one latency range may indicate a direct monosynaptic input, another, a disynaptic input with a location of the interneuron open to question, and a third range possibly indicating pathways via the cerebellum or reticular formation or nucleus of Cajal, etc. In the experiments where decerebellation and/or anaesthesia are employed (Wilson et al., 1966), certain routes from the labyrinth will be eliminated and latency separation into discrete populations will begin to fade.

Wilson et al. (1967) and Peterson (1970) also examined the latency of ipsilateral labyrinth input into the VNC. They found the monosynaptic neurons to have a latency range of 0.8 to 1.5 msec, a finding in agreement with this study. Di- and polysynaptic responses had a latency for example in the MVN of 1.7 to 4 msec (Wilson & Yoshida, 1968). It is to be expected that the range seen in this study exceeds that of Wilson et al. when the possibility of cerebellar reverberations is considered.

Precht & Shimazu (1965) have proposed three possible mechanisms for the production of long latency excitation of VNC units after single shocks of the vestibular nerve: (1) Slow conduction velocity of primary afferent fibres mediating excitation of tonic neurons, (2) synaptic contacts of primary afferents with tonic nueurons differing from those with kinetic ones, (3) multisynaptic activation of VNC units. Polysynaptic responses may also be caused by late synaptic bombardment (Hunt & Kuno, 1959). The fact that small doses of Pentobarbital abolish only these delayed discharges (Precht & Shimazu, 1965) supports the idea of multisynaptic activation of the delayed spikes. Possible anatomical substrates for multisynaptic excitation of vestibular neurons may be either internuncial neurons in the VNC or collaterals of the axons leaving the VNC (Brodal et al., 1962).

The scarcity of inhibitory responses in other studies mentioned is probably due to the cerebellar lesion and the anaesthesia. Our study offers possibilities of inhibitory pathways probably travelling via the cerebellum and the reticular formation. Further support for the notion of different inhibitory pathways was the presence of different latency populations. All inhibitory responses in this study as well as those of Ito et al. (1969), Kawai et al. (1969) and Wilson & Yoshida (1968) are at least disynaptic. Absence of monosynaptic inhibitory input was observed throughout the VNC.

In the present study, excitatory as well as inhibitory responses to contralateral labyrinth stimulation were noted. It cannot be determined whether the inhibited units are of type I and the excited units correspond to type II, as suggested by Shimazu & Precht (1966). The earliest contralateral excitatory activation of the VNC except for the LVN was 1.6 to 2 msec, i.e. within the disynaptic range. This is in agreement with the 1.7 msec described by Wilson et al. (1968). Shimazu & Precht (1966) observed the shortest latency to be 3.2 msec but they were examining solely type II cells. The possible explanation for this discrepancy is that some of the cells in this study and that of Wilson's were not type II, and/or some type II neurons may be monosynaptically activated by commissure fibres (Mano et al., 1968).

It was interesting in the present study to find an absence in the LVN of disynaptic responses to contralateral labyrinth excitatory inflow. Although this is to some extent supported by previous findings (Shimazu & Precht, 1966) it is still questionable, since these indicated an absence throughout the VNC, whereas this study restricted it to the LVN. It is possible that the crossed labyrinthine influences were mediated through extracommissural connections which are consistently excitatory, the LVN known to be particularly receptive to these (Shimazu & Smith,

1971) However, the response latencies seen by these authors were 7–8 msec, whereas in our study the earliest response was at 1.8 msec

Inhibitory responses elicited from the contralateral labyrinth were found throughout the VNC Surprisingly, considering the response latencies to excitatory contralateral input, the area of the VNC showing the shortest response latency to this type of input was the LVN

The SVN, MVN and LVN all contained units receiving contralateral labyrinth inhibitory information via disynaptic pathways, whereas in the DVN they were at best trisynaptic These values compare favourably with those seen by Wilson et al (1968) who found response latencies of 1 6 to 3 7 msec in the MVN However, their latency range was more restricted and the other vestibular nuclei were not examined

Shimazu & Precht (1966) found the inhibitory response latency to be dependent upon the type of neuron recorded Tonic type I neurons had a relatively long latency, 4 msec on average, whereas kinetic type I neurons showed response latencies as short as 2 2 msec, i e within the disynaptic range It has been suggested by Wilson et al (1968) that the difference in latency seen in their study vis a vis that of Shimazu & Precht (1966) was due to stimulating receptors other than those of the horizontal canal which could activate faster pathways Shimazu (1972) on the other hand believes that the latency differences of commissural inhibition depend neither on the different test systems nor on the different receptors concerned but on the functionally different kinds of recipient vestibular neurons

ZUSAMMENFASSUNG

Bei 24 wachen Katzen wurde der Innerohreinfluß auf die Vestibulariskerne untersucht Die Reizung erfolgte durch elektrische Stoße mit Hilfe von zweipoligen Elektroden aus Silberdraht Die Elektroden wurden in n utricularis und n ampullaris lateralis eingestochen In allen Vestibulariskernen wurden Einzelneuronen extrazellul [illegible]
Gebit [illegible]
gisch [illegible]
weitem mehr Neuronen die auf Reize von beiden Labyrinthen antworteten als bei einseitig antwortenden Neuronen Die Unterschiede wechselten mit den individuellen Kernen Sowohl anregende als auch hemmende Reizantworten wurden beobachtet Polysynaptische Reizantworten waren haufiger als mono- oder disynaptische Eine monosynaptische kontralaterale Reizantwort spiegelt genau die Verteilung der ipsilateralen Reizantworten innerhalb jeden Kerns Sowohl die anregenden als auch die hemmenden Reizantworten zerfielen in verhaltnismaßig gut definierte Gruppen beruhend auf der Latenzverteilung Dies bedeutet verschiedene Wege des Innerohreinflusses auf die Vestibulariskerne

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THE INFLUENCE OF LINEAR ACCELERATION ON OPTOKINETIC NYSTAGMUS IN HUMAN SUBJECTS

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Abstract The influence of linear acceleration on optokinetic nystagmus (OKN) was studied in human subjects Linear acceleration was applied to the subjects by means of the parallel swing and also by the transfer of the subjects in one direction either right or left The cortical form of OKN increased the frequency amplitude and eye speed of the slow phase Of the three the increase in eye speed was the most pronounced The subcortical form of OKN was not only increased but was also disturbed by the linear acceleration When the compensatory eye movement with linear acceleration and the slow phase of OKN were in the same direction the nystagmus increased remarkably Contrarily when the two directions were opposed to each other nystagmus was inhibited These results proved that the otolithic organs are not only able to promote but also to inhibit visual function

The study of the relation between vestibular system and optokinetic system is one of the important aspects of equilibrium research It is already known that the two systems are function related in the eye movements (Ohm, 1936, Jung, 1947 De Kleyn 1949 and others) In connection with the two systems, Jung named them Das optisch vestibulare System" and Morimoto (1955) named them the optokinetic vestibular tract Veenhof (1964) and Kitahara (1967) reported that optokinetic nystagmus (OKN) was influenced by linear acceleration The present study was performed in order to clarify further the mechanism of the effects induced by linear acceleration

MATERIALS AND METHODS

The subjects were 20 healthy students, ae 19-21 years The optokinetic stimulation w applied by using an optokinetic cylinder, cm in diameter, 10 cm in height, 2 kg in weight having eight 1 cm wide black stripes on its surface (Fig 1) A convex lens of 4D was placed in front of the subject s eyes to magnify the cylinder, which is motor driven to rotate at a constant speed of 200°/sec The subjects underwent optokinetic stimulation with either attentive or unattentive gaze, according to the theory of Jung (1953) The induced eye movements of the subject were recorded electronystagmographically with time constants of 0 03 sec and 2 0 sec and with paper speeds of 1 cm/sec or 10 cm/sec

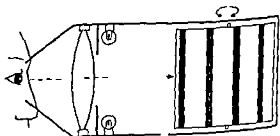


Fig 1 The optokinetic cylinder



Fig 2 Optokinetic cylinder fixed on the chair producing parallel swing

METHODS AND RESULTS

Experiment I

The apparatus for producing parallel swing which is suspended by four steel wires 180 cm in length is shown in Fig 2 The subject was moved right and left manually for a distance of about 40 cm and for a period of 2 8–3 0 sec Therefore the subject who was seated in a chair upon the apparatus received a horizontal linear acceleration to the right and left with a maximum velocity of about 109 cm/sec In this way 20 subjects were tested by applying optokinetic stimulation with or without the linear acceleration

The OKN without linear acceleration was tested first The subject was requested to stare attentively at the movement of the stripes Five seconds after the optokinetic stimulation was commenced the frequency and total amplitude of OKN in the subject were recorded for 10 sec Then the maximum eye speed in the slow phase in the same subject was observed for a succeeding period of 10 sec The absolute values of amplitude of OKN and slow phase eye speed were calibrated from the values obtained with eye movements between two visual points 10° apart The mean value of the R OKN and L OKN was used as the value of OKN After an interval of about 10 minutes the next optokinetic stimulation was applied to the same subject together with linear acceleration Fig 3 shows the OKN without and with linear acceleration in a representative case

The OKN values in the subjects are shown in Table I A statistical analysis of *t* demon

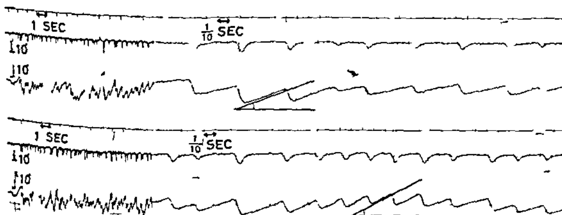


Fig 3 A case of the cortical form of OKN without linear acceleration (*upper*) and with linear acceleration (*lower*) The OKN with linear acceleration is increased in frequency total amplitude and eye speed of slow phase (*lower*)

Table I *Values of the cortical form of OKN without and with linear acceleration*

	Without acceleration		With acceleration	
	Mean value	Standard deviation	Mean value	Standard deviation
Frequency	24.8	4.5	28.3	3.9
Max eye speed of slow phase (deg/sec)	37.4	9.7	56.2	13.3
Total amplitude (mm)	201	54.9	234	93.9

strated that each value of OKN was increased significantly with linear acceleration (Table II) The increase in eye speed during the slow phase was most marked

Experiment 2

Five subjects were tested in this experiment Each subject was ordered to look unattentively at the cylinder described earlier As shown in Fig 4, both increase and inhibition of the OKN were observed in relation to specific phases of parallel swing For example, the subjects with R OKN stated that it was easy to gaze at the movement of the stripes when swinging from left to right, but that it was difficult to look at the movement of the stripes when swinging in the opposite direction

Experiment 3

The effects of linear acceleration on OKN were observed more extensively in this experiment Five subjects were used As shown in Fig 5, linear acceleration was applied to the subject, either to the right or to the left The linear acceleration lasted about 8 9/sec² at a distance of 10 m from the starting point During the test, the subject was ordered to look unattentively at the cylinder Fig 6 indicates the R OKN in a subject As illustrated in Fig 7, pronounced nystagmus is elicited when the compensatory eye movement induced by linear acceleration and the slow phase of OKN are in the same direction Contrarily nystagmus is inhibited when the compensatory

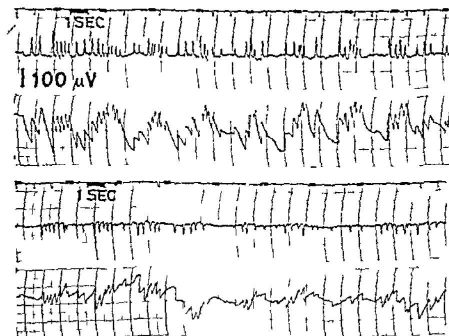


Fig 4 A recorded wave of R OKN (*upper*) and L-OKN (*lower*) in a subject Markers indicate the position where the subject was moved to the left The subcortical form of OKN was alternately promoted or inhibited according to the period of parallel swing

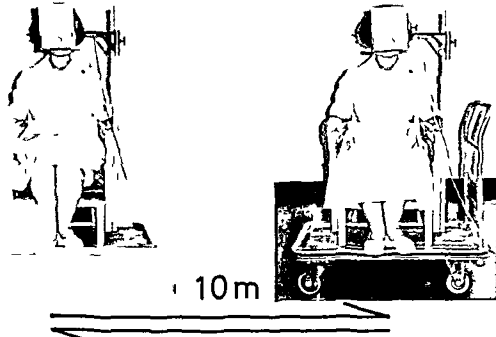


Fig 5 Linear acceleration to the right or left The subject is transferred by the chair with optokinetic cylinder

eye movement and the slow phase are in opposite directions to each other

DISCUSSION

Compensatory eye movement is observed in both humans and animals when they undergo linear acceleration For example, Jongkees & Groen (1946) described how the compensatory eye movement occurred as a result of otolith-ocular reflex Bertrand & Veenhof (1964) recorded the efferent potentials from otolithic stimulation and poved that the eye movement with linear acceleration originated from the otolithic organs Jongkees & Philipszoon (1962, 1963) observed that nystagmus was also induced by applying linear acceleration to rabbits

Nystagmus and nystagmoid eye movements with linear acceleration were observed also by Bergstedt (1961), McCabe (1964), Niven et al (1966), Shirabe (1967), and others From these findings it could be said that the eye movement induced by linear acceleration is already itself in readiness for nystagmus—so to speak, "*Nystagmusbereitschaft*"

Veenhof (1964) reported that the nystagmus in rabbits was easily induced, when otolithic stimulation was superimposed on optokinetic stimulation Also in the present study, the *cortical form of OKN increased with linear* acceleration, when the subject gazed attentively at the movement of the stripes

The cortical optokinetic stimulation might be capable of abolishing the inhibitory effect *resulting from the linear acceleration* used in this experiment Contrarily, both increase and inhibition of OKN were observed alter

Table II *Significant difference in values of cortical form of OKN between absence and presence of linear acceleration*

Frequency	Total amplitude	Max eye speed of slow phase
$|t|=5\ 428$ $>t_{18}$ (0 001)	$|t|-2\ 761$ $>t_{18}$ (0 02)	$|t|-8\ 203$ $>t_{19}$ (0 001)

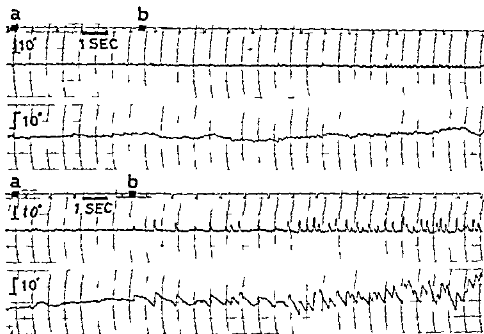


Fig 6 A case of the subcortical form of R-OKN with the transfer to the left (*upper*) and to the right (*lower*) (*a*) indicates the beginning of optokinetic stimulation and indicates the starting of transfer of the subject

nately relating to parallel swing, when the subject looked vacantly at the cylinder In other words, the subcortical form of OKN was not only promoted but also inhibited by linear acceleration As mentioned by the subjects, there were two different phases—ease and difficulty—when looking at the movement of he stripes This fact clearly indicates that the vestibular organs not only support but also interfere visual function

Huizinga & Meulen (1951) proved a close relationship between subcortical OKN and vestibular nystagmus after rotation Similar opinions were already mentioned by Mowrer (1935), Ter Braak (1936) and others According to the report of Jung (1947) the vestibular nystagmus after rotation in the same direction enhanced OKN but detracted from OKN when applied in the opposite direction Fukuda et al (1957) found that the OKN in rabbits increased with subliminal rotatory stimulation Since then Amenomori (1970) has demonstrated that OKN was also inhibited under the condition with subliminal rotation

There is a similarity in the effects of linear acceleration and subliminal rotatory acceleration, nystagmus is produced neither w parallel swing nor by subliminal rotati However, the so called Nystagmusbere schaft eye movement is observed when eit stimulation is applied to the subject

As reported by Hood (1967), the direction

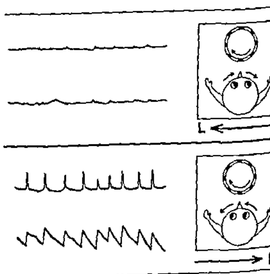


Fig 7 Illustration of the waves shown in Fg Nystagmus is induced remarkably when the slow phase OKN and the compensatory eye movement with l acceleration are in the same direction (*lower*)

subcortical OKN reversed in slow phase as well as in vestibular nystagmus, when the direction of optokinetic stimulation was alternated This indicates, as pointed out by other authors, that the slow phase is fundamental in producing nystagmus

In conclusion, it could be said that the two directions of the slow phase in OKN and of compensatory eye movement induced by linear acceleration might play the most important role in the relation between OKN and linear acceleration

ZUSAMMENFASSUNG

Der Einfluß der linearen Beschleunigung auf den OKN wurde diskutiert Dafür wurde die lineare Beschleunigung an 20 gesunden *Versuchspersonen durch parallel swing* und auch durch den Transport nach links oder rechts appliziert Die foveale Form des OKN nahm dabei in Frequenz Amplitude und Augengeschwindigkeit der langsamen Phase zu Die retinale Form des OKN wurde von der linearen Beschleunigung nicht nur angehoben sondern auch gestört Wenn die kompensatorische Augenbewegung durch die lineare Beschleunigung und die langsame Phase des retinalen OKN die gleiche Richtung hatten nahm der *Nystagmus deutlich zu Wenn andererseits* die beiden Richtungen entgegengesetzt waren wurde der Nystagmus fast nicht ausgelost Aus diesen Resultaten ist ersichtlich daß die Otolithorgane nicht nur die optokinetischen Funktionen fordern sondern auch inhibieren konnen

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THE IMPORTANCE OF POTASSIUM IN THE FUNCTION OF FROG SEMICIRCULAR CANALS

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Abstract The slow potentials and afferent discharge of impulses in frog semicircular canals have been studied at different endolymphatic and perilymphatic K^+ concentrations Results indicate that the presence of K ions in the bathing fluids is essential for maintaining the receptor function in crista ampullaris although very low concentrations of this ion in the perilymph are sufficient to preserve the receptor responsiveness to mechanical stimuli The hypothesis is put forward that K^+ may be pumped from the exterior of the canal towards the intracupular structures where it accumulates A K rich endolymphatic environment does however appear to be necessary to ensure the resting activity of ampullar receptors and their ability to be disfacilitated during inhibitory cupula deflections

K^+ is generally claimed to play an important role in the function of labyrinthine and lateral line organs on the basis of indirect evidence A high K^+ concentration is almost invariably the rule in the endolymph of most animal species (Smith et al 1954 Citron et al, 1956, Murray & Potts 1961 Johnstone et al, 1963, Naito et al 1965 Rossi et al 1973, Sellick & Johnstone, 1975) and has been also evidenced in the intra cupular fluid of the lateral line organ (Russell & Sellick 1976)

However, experiments intended to assess more directly the role of K ions in labyrinthine receptors have given contradictory results For instance, the cochlear function is impaired after substitution of Na for K in the endolymph (Konishi et al 1966) whereas the microphonic potential of the goldfish sacculus is not appreciably affected in the same condition (Matsuura et al, 1971) Also the rece[illegible] in frog crista ampullaris maintained their re[illegible] sponsiveness after replacement of the endolymph with a K low medium such as ordinary Ringer solution (Rapuzzi & Casella 1967 Taglietti et al, 1973, Valli et al, 1974, Valli & Zucca, 1976)

The present study was intended to investigate further the role of K^+ in the sensory function of frog semicircular canals by analysing the effects of different perilymphatic and endolymphatic K^+ concentrations on slow ampullar and nerve potential which reflect the activation process in hair cells and in primary neuron endings respectively (Valli & Zucca 1976, Taglietti et al, 1977)

The experiments required the development of a procedure allowing separate substitution of the endolymph and of the perilymph in isolated canal preparations Stimulation was achieved by producing controlled flows inside in the canals by means of a suitable microinjection device The responses obtained in open canals were compared with those evoked in intact labyrinths by rotatory stimulation

METHODS

The right vertical posterior semicircular canal of frogs (*Rana esculenta* L.) weighing 35–40 g was isolated according to Rapuzzi & Casella

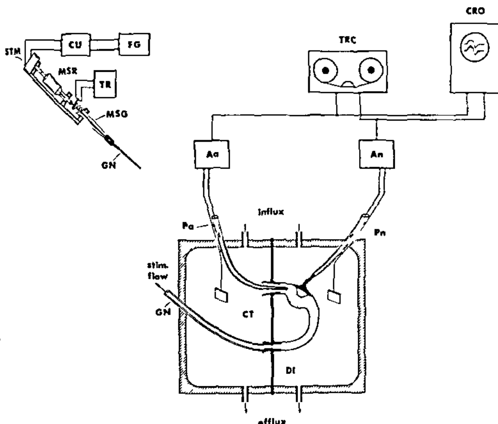


Fig 1 Schematic representation of the experimental set-up *FG* function generator *CU* control unit *STM* stepper motor *MSR* micrometer worm *TR* strain gauge *MSG* microsyringe *GN* glass needle *DI* polyester diaphragm *CT* cone shaped tubes *Pa* and *Pn* lead-off pipettes *Aa* and *An* amplifiers *TRC* tape recorder *CRO* storage oscilloscope

(1967) The mouths of the isolated canal were tightly fixed to two capillary glass tubes sealed in a thin polyester diaphragm separating two compartments (5 ml capacity) in a perspex chamber (Fig 1) The canal s perilymphatic wall and the ampullar nerve were thus bathed in the fluid of one compartment while the canal interior communicated only with the other Stimuli consisted of sinusoidal displacements of the canal fluid of 0 1 Hz frequency and variable amplitudes which were produced by a microsyringe connected via a glass needle to one of the tubes holding the canal The micro syringe plunger (Ø 0 5 mm) was driven by a servo-controlled stepper motor and its move ments (maximum 15 μm peak to peak) were monitored by means of a strain gauge The nerve potentials (slow potentials and neural spikes) were picked up from the whole ampul lar nerve by suction electrodes provided with pressed Ag/AgCl pellets The ampullar potentials were recorded by means of a thin glass pipette (Ø 20 μm) introduced into the ampulla via the utricular opening

Both the fluid electrodes and the stimulating device were filled with the same solution as that in the compartment in which they were immersed The slow potentials were conven tionally amplified 1000× in d c monitored on a storage CRO and recorded in FM on mag netic tape The spikes were further amplified in a separate a c channel and their frequency was evaluated by means of an electronic counter

In order to avoid damaging the preparations the bath level in both compartments was main

tained constant even during replacement of the fluid This was performed, through in–out pipes, by leaving 250 ml of the new solution to flow through the compartments Washing of the canal interior was facilitated by activating the stimulation device during the fluid change Dye experiments proved this procedure to be sufficient to achieve complete replacement of the fluid inside the canals and prevent any contamination between the endolymph and the perilymph

In different groups of experiments the K^+ content of normal Ringer[1] was changed from 0 to 50 mM when replaced for the endolymph and from 0 to 2.5 mM for the perilymph Osmotic pressure in the solutions was kept constant by varying the NaCl concentration

The experiments on the intact labyrinth were performed in isolated frog heads which were fixed to the bottom of a bath at the centre of a small turntable (Taglietti et al, 1977) in such a way that the vertical posterior canal lay in a horizontal plane Stimuli consisted of sinusoidal variations of the turntable's angular velocity (maximum from 10 to 310 deg/sec) having 0.1 Hz frequency, superimposed on a constant rotation (160 deg/sec) Obviously, only replacements of the external id and recordings of the nerve potentials possible in these experiments

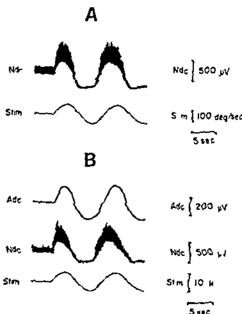


Fig 2 Slow potential recorded in intact labyrinths (A) and in isolated canals (B) at the normal endolymphatic and perilymphatic K^+ concentration (*Adc*) ampullar potential (*Ndc*) nerve potential Stimulus (*Stim*) tracings indicate angular velocity of the turntable in (A) and displacement of the microsyringe plunger in (B) Upward deflections indicate positive potentials for the nerve and negative potentials for the interior of the ampulla

RESULTS

In Fig 2 the slow responses obtained in isolated open canals at the normal endolymphatic (50 mM) and perilymphatic (2.5 mM) K^+ concentration (Rossi et al 1973) are compared with those evoked in the intact labyrinth by rotatory stimulation The potentials recorded in the two different experimental conditions are similar in terms of amplitude and time course Moreover, it should be noted that the excitatory and inhibitory phases of the slow potentials reach approximately the same absolute peak amplitude, referred to the base line This indicates that, under normal conditions, the slow potentials can be driven in an equivalent range above and below the resting level

The nerve tracings in Fig 2 reveal that a sustained spike discharge is present at rest which grows and fades according to the excitatory and inhibitory phase of the stimulus

Effects of K^+-deprivation

The tracings in Fig 3 illustrate the effects of complete withdrawal of K^+ from the fluids bathing the interior and the exterior of the canal It may be seen that the responses fall progressively and disappear completely in about 60–80 min This is quantitatively illustrated in the histograms in the same figure where the peak to peak amplitudes of the slow potentials are plotted versus treatment time over a number of experiments No significant differences could be evidenced between the

[1] Composition NaCl 117 mM KCl 2.5 mM $NaHCO_3$ 1.2 mM NaH_2PO_4 0.17 mM $CaCl_2$ 1.8 mM

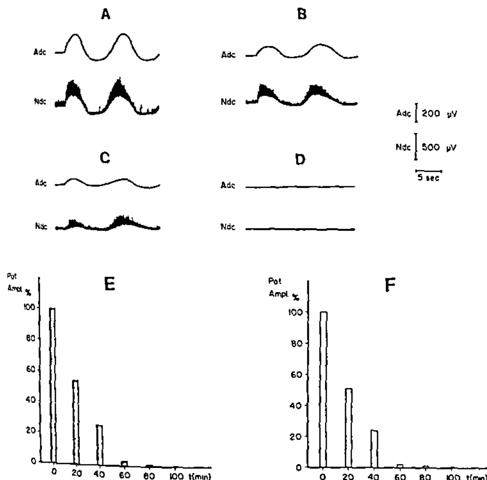


Fig 3 Effects of endolymphatic and perilymphatic K free solutions on slow ampullar (*Adc*) and nerve (*Ndc*) potentials Recordings 10 min (A) 20 min (B) 40 min (C) 80 min (D) after exposure to the K free solutions Histograms the amplitude of ampullar (E) and nerve (F) potentials (mean values from 5 experiments) are expressed as percentages of controls

behaviour of nerve and ampullar potentials, which were affected in a similar way by changes in K^+ concentration in the fluids Since this parallelism was constantly observed in all the experiments of this study, only the tracings of nerve potentials will be presented in the following figures

The strong depression of the receptor function produced by the complete withdrawal of K^+ both in the endolymph and in the perilymph was not observed when this ion was removed only from one of this fluids (Fig 4) In this condition preservation of the responses actually depends on the amount of K^+ present in the other fluid However as illustrated in the graphs in Fig 5, the receptor function was more easily impaired by reducing the endolymphatic rather than the perilymphatic K^+ The greater effectiveness of external K^+ in maintaining receptor function clearly emerges when it is seen that a complete receptor block occurs at internal K^+ concentrations of about 20 mM, whereas the same effect requires the external K^+ to be reduced to about 0 25 mM

The slow potentials can only partially be restored by reinstating the normal K^+ concentration in the fluids, when they have been suppressed by protracted treat t w K-

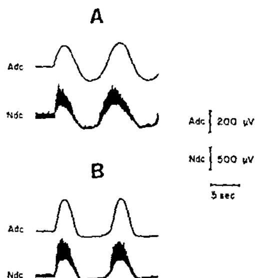


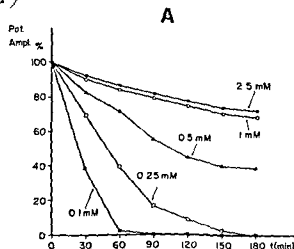
Fig 4 Effects of perilymphatic (A) or endolymphatic (B) K free solutions on slow ampullar (*Adc*) and nerve (*Ndc*) potentials 90 min after replacement of fluids

free solutions Irreversible alterations are therefore likely to arise in the sensory organ during prolonged K^+ deprivation

Effects of endolymphatic K^+ reduction on the excitatory and inhibitory responses

As reported above the responsiveness of ampullar receptors is preserved to some extent when K^+ is withdrawn from the endolymph only However, certain modifications in the responses occur in this condition mainly a lowering in the amplitude of the inhibitory phase of the slow potentials whereas the excitatory phase remains unchanged or even enhanced (Fig 4B) Moreover, as Fig 6 shows *this reduction in the inhibitory phase is invariably associated with a parallel decrease in the* spontaneous discharge of the receptors The modifications in the receptor responses as a function of the endolymphatic K^+ content are illustrated in the histograms in Fig 7 which also shows the responses in the intact labyrinth

The selective depression in the inhibitory phase of the slow potentials induced by low endolymphatic K^+, results in an abnormal distortion in the conversion process This distortion is clearly evidenced in the graphs in Fig 8 where the receptor transduction characteristics are drawn as obtained from the peak values of the slow nerve potentials in response to sinusoidal stimuli of different amplitudes At the normal endolymphatic K^+ concentration, the conversion curve in isolated canals appears to be linear to a considerable extent both in the excitatory and inhibitory range and virtually duplicates that recorded in the intact labyrinth under rotatory stimulation By contrast, *in the absence of internal* K^+, the pres



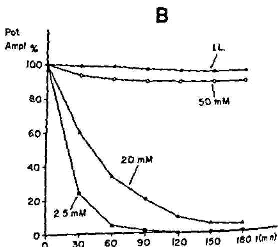


Fig 5 *(A) Amplitude of slow nerve potentials as a function of the perilymphatic* K^+ content The endolymph was K free (B) Amplitude of slow nerve potentials as a function of the endolymphatic K^+ content The perilymph was K free *I L* intact labyrinth Values (means from 5 different experiments) are % variations over treatment time

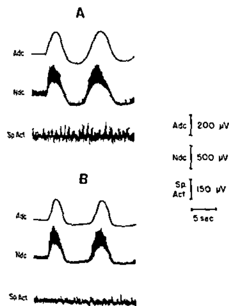


Fig 6 Ampullar potentials (*Adc*) nerve potentials (*Ndc*) and spontaneous activity (*Sp Act*) recorded under normal conditions (A) and 90 min (B) after replacement of the endolymph with a K free solution The perilymph was normal

ence of a marked knee at the 0 point on the curve reveals an abnormal rectification in the conversion process

DISCUSSION

The experiments clearly show that the mechanism supporting the receptor function in frog semicircular canals cannot be activated when K^+ concentration both in the internal and in the external fluid is reduced below a critical value However, the minimum concentration of this ion which has to be present in at least one of the fluids to ensure receptor responsiveness to mechanical stimuli, is much higher in the endolymph than in the perilymph This could indicate that the perilymph is the most accessible source of this ion for the hair cells of crista ampullaris It is therefore likely that the function of ampullar receptors is sustained by a process whereby K^+ is pumped from the exterior towards the interior of the canal

A K pumping process, responsible for the high K^+ content of the endolymph has been evidenced by Dohlman & Radomski (1968) in pigeon canals and by Sellick et al (1972) in guinea pig utricles while some data (Dohlman et al, 1959, Bairati & Iurato, 1960, Flock 1967, Smith 1970) suggest that the pumping process may be located in some auxiliary structures of the sensory organs

If one accepts that a high K^+ concentration at the hair bearing pole of the sensory cells is essential for their function, the extreme resistance of receptor responsiveness to withdrawal of this ion from the endolymph, provided that K^+ is available in the perilymph, suggests that K^+ may not only be pumped inward but also stored in the mucopolysaccharidic cupular structures In fact, intracupular accumulation of K^+ is not unlikely since mucopolysaccharides are known to display a considerable cation affinity Moreover ion diffusion from the jelly like cupular substance towards the endolymph probably takes place at a rather slow rate These mechanisms could maintain a potassium concentration at the inner pole of the hair cells sufficient to preserve their function even after several hours of endolymphatic K^+ deprivation This hypothesis is strongly supported by the recent observation that the cupulae of free standing neuromasts can maintain a K rich microenvironment above the sensory epithelium (Russell & Sellick 1976)

It is more difficult to interpret the changes in receptor conversion seen when the endolymphatic K^+ is reduced In fact, over a wide range of K reduction, a steady condition may be reached where the resting discharge and the inhibitory responses are almost suppressed whereas the receptors still respond normally to excitatory stimuli

The basic mechanism proposed by Davis (1965) for hair cell activation postulates the existence of a resting cation current across the mechano-sensitive cells, which is modulated by impedence variations in their hair bearing membranes, and some evidence (Konishi et al, 1966, Russell & Sellick 1976) suggests that K ions may carry the receptor potential current According to this mechanism a reduced

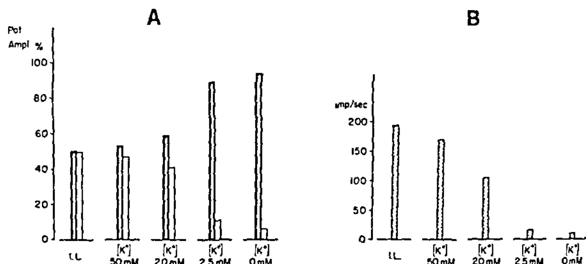


Fig. 7. (A) Amplitude of the excitatory (▨) and inhibitory (□) peaks of slow nerve potentials (referred to the base line) as a function of the endolymphatic K^+ content. The perilymph was normal. Values are expressed as percentages of the peak-to-peak potential amplitude. (B) Mean frequency of the receptor resting discharge as a function of the endolymphatic K^+ content. *I.L.* intact labyrinth. Measurements were made 90 min after replacement of the endolymphatic fluid.

K^+ concentration in the fluids will only result in a reduced availability of current carriers. This could explain the depression observed in the receptor's resting discharge and thus their reduced ability to be 'disfacilitated' during the inhibitory phase of the stimulus, but can in no way account for the concomitant preservation of the excitatory responses. The hypothesis may therefore be put forward that mechanically-evoked excitation in hair cells is sustained by a mechanism to some extent distinct from that responsible for their resting discharge. The possible existence of these distinct mechanisms in ampullar receptors is also supported by the observation that, under suitable conditions, curare can depress mechanically evoked discharges without affecting their spontaneous activity (Valli et al., 1974).

The mechanisms sustaining receptor function in labyrinthine organs are probably more

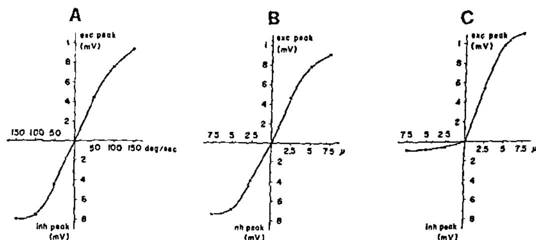


Fig. 8. Conversion characteristics between stimulus intensities and slow nerve potentials in intact labyrinths (A) and in isolated canals both at the normal endolymphatic K^+ concentration (B) and after replacement of the endolymph with a K free solution (C). Perilymphatic K^+ was normal. Values (means from 5 experiments) were determined 90 min after replacement.

complex than those postulated in Davis' hypothesis and some other process viz , the K-dependent mechanosensitivity of biological membranes (Tasaki, 1960) and ' displacement potentials due to mechanical deformation of the cupular mucopolysaccharidic molecules (Christiansen, 1964, Barrett, 1975), may play a role in the transduction process taking place in labyrinthine sensory organs

Finally it should be pointed out that slow ampullar and nerve potentials invariably display similar behaviour to K^+ reductions in the fluids This confirms the view that K ions are involved chiefly in the earlier stages of receptor activation rather than in the subsequent links leading to afferent discharge in the nerve fibres

ZUSAMMENFASSUNG

Die langsamen Potentiale und Impulsentladung die im sensorischen Organ der Bogengange des Frosches auf treten wurden bei verschiedenen endolymphatischen und perilymphatischen K^+ Konzentrationen analysiert Die Ergebnisse zeigen daß das Auftreten von K Ionen in den Flussigkeiten für die Aufrechterhaltung der Ampullarrezeptorfunktion unerlaßlich ist daß jedoch sehr geringe Konzentrationen in der Perilymphe ausreichen um d e Erwiderung der Ampullarrezeptoren auf die mechanische Stimulierung zu gewahrleisten Es ergibt sich d e Vermutung daß die K Ionen von der Außenflussigkeit in die intrakupularen Strukturen gepumpt werden wo s e sich summieren Eine hochkonzentrierte endolymphatische K Umgebung erscheint jedoch fur die Gewahrung der Ruheaktivitat der Ampullarrezeptoren unerlaß l ch

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RESPONSES OF SQUIRREL MONKEY VESTIBULAR NEURONS TO AUDIO FREQUENCY SOUND AND HEAD VIBRATION

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Abstract A study was made of the response of peripheral vestibular neurons in the squirrel monkey to head vibration and air borne sound in the frequency range from 50–4 000 Hz Responses were measured in terms of the phase locking of discharge and changes in firing rate The lowest phase locking thresholds for vibration were −70 to −80 dB re 1 *g* and median values in the most sensitive frequency range (200–400 Hz) were −20 to −40 dB re 1 *g* the minimum and median thresholds for sound were 76 and 120–130 dB SPL respectively Rate-change thresholds were 10–30 dB above phase locking thresholds The squirrel monkey sacculus has no special sensitivity to vibration in comparison with the other vestibular end organs the median phase locking threshold to sound of ... ular neurons exceeded 100 dB SPL Irregularly discharging neurons are more sensitive than regularly dis...ing units Evidence is presented that the response to intense sound involves a hair cell mechanism

Questions concerning the sensitivity of the mammalian vestibular endorgans to air borne sound and head vibration have not been satisfactorily answered Intense sound and vibration have been reported to produce vestibular reflexes and illusions of movement (von Bekesy, 1935, Parker et al 1975 Lackner & Graybiel, 1974) The etiology of these reactions is unclear Mammalian vestibular receptors respond to sound stimuli after fenestration of a semicircular canal (Mikaelian 1964) But with the exception of one study (Trinker & Partsch, 1959) responses to sound (Mikaelian, 1964, Katsuki & Davis, 1954) or vibration (Adrian, 1943) have not been observed in the intact mammalian vestibular labyrinth The sensitivity of the sacculus is of particular interest The bulk of physiological and anatomical evidence indicates that the sacculus serves a vestibular function in mammals (Lorente de Nó, 1933, Fluur & Mellstrom, 1970, Fernandez & Goldberg 1976a) Nevertheless ablation of the mammalian sacculus has not led to striking vestibular deficits (Versteegh 1927, Igarashi & Kato, 1975) In addition, behavioral and physiological studies strongly indicate that the sacculus functions in part as a hearing organ in a number of non mammalian species (fish, Popper & Fay, 1973 rays, Lowenstein & Roberts, 1951, toads Moffat & Capranica 1976) Evidence of this kind has led to suggestions that the mammalian sacculus has retained a function in hearing To clarify these issues we were interested in determining the stimulus levels at which afferents from the different vestibular endorgans respond to sound and vibration

This information is of interest for other reasons The upper frequency limit of the mechanical response of the mammalian oto-

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liths has not been determined, but is thought to be in the audio-frequency range (Goldberg & Fernández 1975), in contrast, the presumed mechanical response of the semicircular canals to head rotations is limited to frequencies well below this range (Fernandez & Goldberg 1971) Thus, one might expect the otolith organs to be more sensitive to audio-frequency stimuli than the canals There are two uncertainties, however First, it is not clear that activation of vestibular receptors by sound and vibration would have the same mechanical basis as does the response to more physiological head movements Second, mechanical factors are not the only determinants of response dynamics In their response to sinusoidal natural stimuli of 1–10 Hz, vestibular nerve fibers can show a frequency-dependent increase in gain over that predicted from the mechanics of the sensory endorgans (Fernandez & Goldberg, 1971, 1976*b*) The effect is more conspicuous in afferents characterized by an irregular spacing of action potentials, than in units whose discharge is regular If this high frequency gain enhancement were to continue out to audio frequencies, then predictions based solely on mechanical considerations would probably be untrue, particularly since irregularly discharging fibers are more prevalent among canal afferents (Fernández et al , 1972)

METHODS AND MATERIALS

Animal preparation identification of single units

Adult squirrel monkeys (*Saimiri sciureus*) were anesthetized with sodium pentobarbital (15 mg/kg) Single unit activity of vestibular nerve fibers was studied in 9 animals Surgical and recording procedures have been described previously (Goldberg & Fernandez, 1971*a*)

The animal was mounted on a platform which could be tilted and rotated Neurons innervating any particular semicircular canal were easily identified by their response to angular accelerations in various planes Units were considered to be otolith neurons if they proved unresponsive to angular acceleration but were sensitive to head tilt The anatomical organization of the vestibular nerve permits a further classification of otolith neurons The inferior branch of the nerve contains fibers innervating the posterior semicircular canal and the posterior two-thirds of the sacculus whereas the superior branch contains fibers innervating the horizontal and superior semicircular canals, the utriculus, and the anterior third of the sacculus (Gacek, 1969) Otolith neurons were assigned to the inferior nerve if they were encountered in electrode tracks in association with afferents innervating the posterior canal and were assigned to the superior nerve if they occurred in tracks with horizontal- and superior canal afferents The directional selectivity of otolith neurons classified in this way supports the notion that inferior nerve otolith fibers arise from the sacculus and suggests that superior nerve otolith afferents largely originate in the utriculus (Fernandez & Goldberg, 1976*a*) For the purpose of this paper, the designations superior canal, horizontal canal, posterior canal, sacculus, and superior nerve otolith will be used

Fibers were also classified in terms of the regularity of their background discharge The coefficient of variation (standard deviation divided by the mean) of the interspike interval distribution was computed for each fiber Fibers with coefficients less than 0 1 were termed regular , those with coefficients greater than 0 1, ' irregular The activity used was obtained when the head was in an approximately horizontal position

Sound and vibrational stimuli

Vibrational stimuli, generated by a dynamic loudspeaker driver (University model ID 30), were delivered to the frontal bone at the mid line via a plexiglass rod cemented to the driver s diaphragm The amplitude of the vi

bration was measured with an accelerometer (Bruel & Kjaer model 4333) coupled by a hand held probe to a point on the skull located mid way between the occiput and the ipsilateral external auditory meatus Acceleration read ings, taken in three roughly orthogonal directions, were used to compute the total skull acceleration Vibration amplitudes will be expressed as dB re 1 *g* (where *g* is the acceleration of gravity) Calibrations were obtained in only 4 animals Results for the other animals are expressed in terms of the median of these four calibrations The vibrator also produced a significant sound field as measured in the ear canal This sound field was compared with the stimulus levels resulting in single unit responses to head vibration and air borne sound The comparisons indicated that, of the two vibrator related stimulus components, the ac tual head displacements were 20–60 dB more effective than was the incidental sound field

Sound stimuli were generated by a dynamic earphone (Koss model 102) connected to a speculum sealed into the ear canal Sound pressure levels in the ear canal were measured with a calibrated probe tube and condenser microphone (Bruel & Kjaer model 4134), the measurements will be expressed as dB re 0002 μbar (i e dB SPL) The animal was placed in a quiet room whose total ambient noise level measured in a band from 0 05–4 0 kHz was 60 dB SPL, this was well below the sound level which evoked responses in the vestibular nerve

The only stimuli used were sinusoids at 13 standard frequencies spaced in approximately half octave steps between 0 05–4 0 kHz They were usually presented for a period of 3 sec separated by 7 sec of silence five repetitions of each stimulus were normally presented Stimuli were gated on and off with a time con stant of 10 msec Stimulation at each fre quency was begun at maximum levels and was then lowered in 10 dB steps until no response was detected from the unit Maximum vibra tion levels were on the order of −20 dB re 1 *g* maximum sound levels 120–130 dB SPL

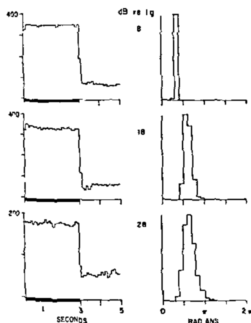
Data analysis

Two principal displays of a unit s responses were used the response histogram which estimates the unit's discharge rate as a function of time during and between stimuli, and the phase histogram which shows the unit s firing probability as a function of the sinusoidal stimulus phase, i e , the tendency of the unit to discharge preferentially over a restricted por tion of the stimulus cycle The latter will be called "phase locking" Response phase was referenced to the electrical signal delivered to the earphone or vibrator

The sensitivity of vestibular nerve fibers was assessed by computing a *phase locking threshold*, defined as the stimulus level at which the fundamental component of the re sponse determined by Fourier analysis equaled 20% of the background rate, and a *rate change threshold*, defined as the stimulus level giving a 20% increase in discharge rate over the background rate The thresholds were obtained by linear interpolation between adjacent stimulus levels, one of which was above threshold, the other below The term 'threshold' is used advisedly here, since our units probably did not show a true threshold in the sense of a stimulus level below which there was no response and above which a re sponse appeared At low stimulus levels, the amplitude of the fundamental component of the response was in most units approximately linearly related to a linear measure of stimulus amplitude Thus the phase locking threshold as defined above, reflects the slope (or gain) of the unit s input–output function

RESULTS

Responses to both head vibration and sound were seen in units from all five endorgans Fig 1 shows results from a horizontal-canal unit responding to 350-Hz head vibration Re sponse and phase histograms obtained at six vibration levels from −58 to −8 dB are in cluded At −58 dB, phase locking was ob served As the stimulus level increased the



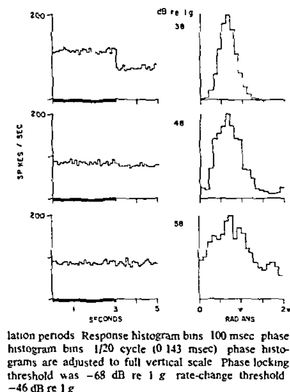


Fig 1 Responses to 350-Hz vibration horizontal-canal fiber Responses at different stimulus levels given by numbers (in dB re 1 *g*) between pairs of histograms In each pair response histogram is at left phase histogram at right For response histograms heavy lines mark stimulation periods Response histogram bins 100 msec phase histogram bins 1/20 cycle (0 143 msec) phase histograms are adjusted to full vertical scale Phase locking threshold was −68 dB re 1 *g* rate-change threshold −46 dB re 1 *g*

phase locking became more precise, until at -8 dB almost all the spikes occurred within one bin of the 20 bin phase histogram A significant increase in discharge rates during the stimulus was also observed at levels greater than −48 dB

Phase locking was always seen at lower intensity levels than were changes in discharge rate Rate change thresholds were obtained in 26 sensitive units, phase locking thresholds averaged 21 5 dB below rate change thresholds (range 10 9 to 28 6 dB) Since rate

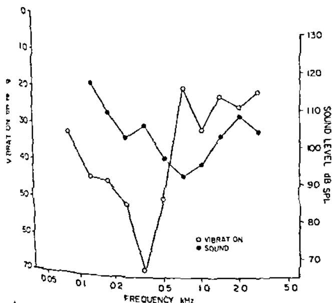


Fig 2 Phase locking thresholds for a horizontal-canal fiber plotted versus frequency as in Fig 1

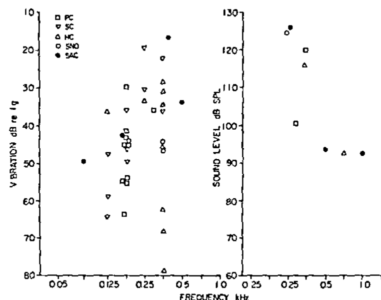


Fig 3 Phase locking thresholds at best frequency for vibration (*left plot*) and sound (*right plot*) See key for the endorgan innervated by each fiber *PC* posterior canal *SC* superior canal *HC* horizontal canal *SNO* superior nerve otolith *SAC* sacculus

change thresholds could not be determined for most units, phase locking thresholds were used as the measure of sensitivity Unless stated otherwise, all references to threshold in this paper will mean phase locking threshold

The unit shown in Fig 1 was typical in two respects First, the rising and falling phases of the responses are abrupt (<100 msec) Second, there is little adaptation of the response during the 3 sec stimulus Perstimulus response declines were usually not seen even when the vibrational stimulus was maintained for 20 sec The response to the 20 sec stimulus, measured as the discharge rate minus the background rate, was studied in 11 units Adaptive effects were considered significant only if there was at least a 10% change in response from the beginning to the end of stimulation Only three of the 11 units showed a significant response decline whereas six units were characterized by an essentially constant response In the remaining two units both of them canal neurons, the discharge changed abruptly at stimulus onset and then exhibited a smaller and more gradual increase as stimulation was prolonged A possibly related pheno-

Table I *Median phase locking thresholds (dB re 1 g or dB SPL)*

Vibration thresholds are shown for all fibers and for canal and otolith fibers separately Sound thresholds are shown for all units and saccular units only Numbers in parentheses are the numbers of responding and non responding units respectively Values are only given if data were available for at least 5 units

Frequency (Hz)	80	125	177	250	350	500	707	1 000
Vibration								
All Units	25 (8 5)	−26 (27 15)	36 (22 13)	−23 (50 27)	−22 (44 24)	−11 (36 15)	−1 (11 5)	4 (11 10)
Canals	22 (7 5)	−27 (21 12)	−41 (19 2)	−28 (40 11)	−25 (32 11)	−10 (22 14)	−2 (10 3)	>3 (6 10)
Otoliths	–	24 (6 3)	>−20 (3 11)	>−21 (10 16)	>−20 (12 13)	−13 (14 1)	–	−8 (5 0)
Sound								
All Units	–	–	>130 (6 9)	>118 (12 16)	121 (17 14)	–	122 (5 3)	–
Sacculus		–	–	110 (6 1)	106 (7 0)	–	–	–

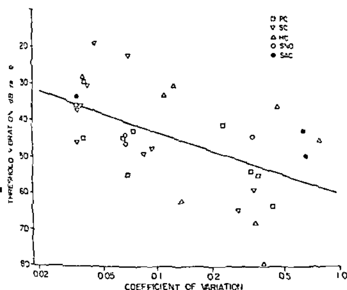


Fig 4 Scatter plot of best frequency vibration threshold (θ in dB) versus coefficient of variation (CV) the latter plotted logarithmi

16 6 $\log_{10}$ CV, significance ... tailed t test)

menon was seen in some units on stimulus termination When the vibrator was turned off, there was an abrupt but incomplete return of the discharge towards background levels followed by a smaller decline requiring 5–20 sec This post stimulus persistence of response was observed in four of the eight units whose perstimulus response increased or stayed constant Of the four, one was an otolith neuron, the remainder canal neurons

Fig 2 plots phase locking thresholds versus frequency for one unit, tuning curves are presented for both vibration and sound As in most units, sensitivity was maximal at one frequency (the so-called best frequency) and declined more rapidly for higher than for lower frequencies There was some tendency for the response to sound to be tuned to somewhat higher frequencies than were the responses to vibration, this is seen both in the turning curves for individual units (Fig 2) and in the distributions of best frequencies for all units (Fig 3) No units were encountered with best frequencies which exceeded 500 Hz for vibration or 1000 Hz for sound Responses to either kind of stimulus were only occasionally seen at 2 8 kHz and were never observed at 4 0 kHz

Fig 3 shows the distribution for the various endorgans (see key) of best frequency thresholds for vibration (left plot) and sound (right plot) Note that units from the sacculus have, if anything, higher vibration thresholds than do those innervating semicircular canals Only one unit responded to sound at any level below 90 dB, this unit (from the sacculus) had a phase-locking threshold of 76 dB SPL at 250 Hz Its best frequency was not determined

Some idea of the profile of activity within the vestibular nerve is obtained from Table 1, where median phase locking thresholds are presented for various afferent populations Also included are the numbers of responding and nonresponding units at each stimulus frequency Again it will be seen that otolith units are less sensitive to vibration than canal fibers, except possibly at the higher frequencies Separate medians for the response of canal afferents to sound could not be computed because fewer than half the units reached threshold at maximum available stimulus levels In contrast, almost all saccular afferents were affected by sound stimulation median thresholds at 250 and 350 Hz are between 106–110 dB The corresponding values for canal units were at least 120 dB at these frequencies

A number of properties of vestibular nerve fibers have been correlated with the regularity of their discharge (Fernández & Goldberg,

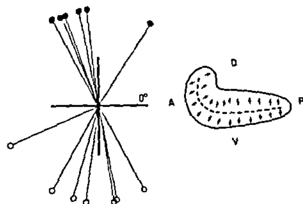


Fig 5 (*Left*) Phase of the fundamental component of the response to 350 Hz sound 13 sacculus units from one animal Phase for each unit is shown as a vector horizontal axis 0° re electrical signal to earphone Units were classified as having upwardly pointing (O) or downwardly pointing (●) functional polarization (*Right*) Morphological polarization map of the sacculus *A* anterior *D* dorsal *P* posterior *V* ventral

1976*b*, Goldberg & Fernandez, 1971*b*) In particular, in their response to sinusoidal natural stimuli below 10 Hz, irregular fibers show a more pronounced high frequency gain enhancement than do regular fibers This difference, it would appear, extends to the audiofrequency range Fig 4 shows a scatter plot of vibration threshold at best frequency versus the coefficient of variation (CV) of background discharge for a group of 34 units Irregular fibers (CV>0 1) have lower thresholds than regular fibers (CV<0 1) This last observation may help to explain why the otolith organs are less sensitive to vibration than are semicircular canals As described in previous work (Fernandez et al 1972) otolith neurons tend to be more regular than canal neurons and, hence, might be expected to have higher vibration thresholds

Fig 5 provides evidence that the response to intense sound involves activation of the hair cells, rather than a direct mechanical action on the nerve fibers A schematic morphological polarization map for the sacculus is shown at the right Approximately half the hair cells have upwardly directed morphological polarization vectors and the other half have their vectors pointing downwards If the stimulus acts via the hair cells, nerve fibers connected to these two groups of hair cells should respond approximately 180° out of phase from one another This assumes that the wavelength of the stimulus is long compared to the size of the macula Stimulation by a mode other than through the hair cells would not be expected to give this result In the graph at the left, the responses to 350 Hz sound of all saccular afferents studied in one animal are shown, each unit is represented by a vector whose angle corresponds to the phase lag of the fundamental component of the response Static tilts were used to determine the functional polarization of individual afferents On this basis, the units have been divided into two groups (for details, see Fernandez & Goldberg, 1976*a*) Units with downward polarization (filled circles) were characterized by a maximum background discharge when the head was in the normal (upright) position those with upward polarization (unfilled circles) showed a minimum discharge in this position As seen in the figure the responses for the two groups were approximately 180° apart The results are consistent, therefore, with the hypothesis that stimulation by sound takes place by a displacement of sensory hair bundles The general similarity of sound and vibration responses obtained from both canal and otolith afferents suggests that this conclusion can be extended to the response of all the vestibular endorgans to audio frequency stimuli

DISCUSSION

The squirrel monkey sacculus is, if anything less sensitive to vibrational stimuli than are the semicircular canals Saccular afferents, though somewhat more responsive to sound than other vestibular fibers, still had median phase locking thresholds above 100 dB SPL Most likely, phaselocking at audio freq would not, by itself, be recognized tivation of the vestibular app changes were required, then threshold would exceed 120

leading to discomfort or pain in humans These considerations, together with the observation that virtually all saccular afferents respond to head tilts (Fernandez & Goldberg, 1976*a*) imply that the mammalian sacculus functions mainly, if not solely, as an equilibrium organ

Intense pure tones have been reported to produce nystagmus in rhesus monkeys, the most effective frequencies were between 200 and 500 Hz where levels above 138 dB were required (Parker et al , 1975) This value is above our median phase locking threshold at 350 Hz, but corresponds fairly well with the estimated median rate change threshold of 142 dB at this frequency

Application of vibration to the head of human subjects was found (Lackner & Graybiel, 1974) to give rise to nystagmus, relatively weak sensations of rotatory self movement, and oculogyral illusions The effects occurred over a range of frequencies (120–280 Hz) approximately the same as those to which our units were most sensitive, higher frequencies were not investigated in the human studies It seems likely that Lackner & Graybiel's results represent a functional correlate of the responses we have been studying One puzzling aspect of their study is that they observed a nystagmus which continued beyond the cessation of the stimulus but did not change sign (although the subjective sensation of movement did change sign) Possibly this observation may be related to the poststimulus persistence of response found in some of our units

It is not surprising that the otolith organs respond to audio frequency stimulation since the bandwidth of their mechanical response is thought to extend to near 500 Hz (Goldberg & Fernandez 1975) A similar sensitivity might not be expected in canal afferents since their response to angular accelerations is band limited to frequencies below 0 1 Hz (Fernandez & Goldberg 1971) These low frequency dynamics are thought to reflect the mechanics of the cupula–endolymph system and principally, the viscous resistance encountered by the endolymph as it moves through the membranous duct The high-frequency sensitivity of our responses makes it unlikely that audio frequency stimulation of canal afferents involves the flow of endolymph Instead, one is led to consider mechanisms based on the distortion of the bony labyrinth and the inertia of the structures in the ampulla, mechanisms similar to those suggested for stimulation of the cochlea by bone conduction (Tonndorf et al , 1966) This hypothesis could also explain the similarity in best frequencies of canal and otolith afferents The units' tuning properties, rather than reflecting the quite different dynamics of the cupula–endolymph system and the otolithic membrane, would then be determined largely by the mechanics of bone conduction These mechanics should be nearly identical for the two sets of endorgans

ZUSAMMENFASSUNG

Es wurde die Reaktion der peripharen vestibularen Neuronen von Affen (*Saimiri*) bezuglich Kopfschwingungen und Schallschwingungen mit Frequenzen zwischen 50–4000 Hz untersucht Die Reaktion wurde bezuglich des Phasenverhaltens der Entladung und der Reiz auf die Anderungen der Frequenzrate hin gemessen Die unterste Schwelle für Phasenbeziehungen bei Schwingungen war –70 bis 80 dB re 1 *g* und der Mittelwert im empfindlichsten Frequenzbereich (200–400 Hz) war –20 bis –40 dB re 1 *g* die unterste bzw mittlere Schallschwelle war 76 bzw 120–130 dB SPL Die Schwelle fur Haufigkeitsanderungen war 10–30 dB oberhalb der Schwelle für Phasenbeziehungen Verglichen mit anderen vestibularen Endorganen hat der Sacculus des Affens keine spezielle Empfindlichkeit des Sacculus Neuronen für Vibrationen und die mittlere Schwelle für Phasenbeziehungen uber schreitet 100 dB SPL Unregelmaßig entladende Neu

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— 1976*a* Physiology of peripheral neurons innervating otolith organs of the squirrel monkey I Response to

available even concerning the cytochemistry of mammalian inner ear sensory cells except for what can be deducted from morphological studies and staining properties Biochemical alterations precede morphological changes and detection of such alterations must thus be regarded as vital especially in connection with cytotoxic effects of antibiotics

Cellular content of ribonucleic acid (RNA) is known to be a sensitive indicator of the functional state in nerve cells (Hyden 1960, Edstrom 1964 Jarlstedt 1966) Since RNA can be determined with great accuracy on the single cell level as shown by the above studies such determinations were used in the present study where the short term effects of gentamicin on RNA content in sensory and ganglionic cells is studied The effect of the drug on the biochemical parameter is compared with morphological changes in sensory cells from the same material

MATERIAL AND METHODS

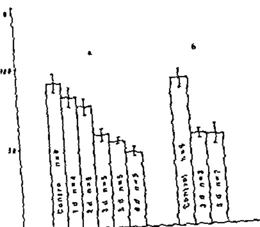
Healthy lizards belonging to the species *Calotes versicolor* were used The animals were kept in a humid environment at about 27°C The sensory cells of the basilar papilla (i e the hearing organ) and ganglionic cells of the acoustic ganglion were investigated in animals given large doses of gentamicin (Schering GMC 3 M-675) Untreated controls were processed and examined in the same way as the treated ones The treated animals were divided into two groups The animals of the first group were given daily intraperitoneal injections of 150 mg gentamicin per kg bodyweight per day The injections were given for 1 2 3 5 or 6 days The animals were sacrificed one day after the last injection The intention with this group was to study the direct drug effects on the content of cellular RNA To study the persistent effects the animals of the second group were given gentamicin in the same way as in the first group for 3 or 5 days The animals were then left untreated for 21 days and subsequently sacrificed After sacrifice of the animals the skull was rapidly dissected and the inner ear structures removed The specimens were either processed for RNA analysis or transmission electron microscopy For the morphological investigation animals were also given the drug for 7 days and subsequently sacrificed the day after the last injection

Cytochemical procedures

The basilar papillae and adjacent structures were placed in Carnoy s solution (i e absolute ethanol chloroform glacial acetic acid 6 3 1 by volume) and fixed for 60 min at 20°C The papillae and surrounding tissue were embedded in paraffin and orientated so as to allow cross sections of 25–30 μm slices Individual sensory cells and acoustic ganglionic cells were isolated by micromanipulation under a phase contrast microscope and the RNA subsequently extracted with ribonuclease in an oil chamber The collected extracts were evaporated on a quartz glass and redissolved in a glycerol-containing buffer to form lens shaped drops The drops were photographed in ultraviolet light at a wavelength of 258 mμ together with a reference system The amounts of RNA in the ultraviolet absorbing spots were determined by a photometric system (Edstrom 1964) Determinations were carried out on samples of either 5 sensory cells or 2 ganglionic cells according to the minute amounts of RNA in the cells

Electron microscope procedures

The specimens intended for morphology were fixed in 1% OsO_4 in phosphate buffer pH 7 4, for 2 hours and processed for transmission electron microscopy according to usual methods (Rhodin 1954, Luft 1961 Reynolds 1963) They were then sectioned on a LKB ultramicrotome and mounted on one hole copper grids and finally examined in a Jeol 100 C electron microscope



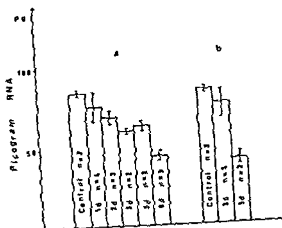
ig 1 RNA content ±S E M in sensory cells from the . . . after the last injection P values for the significance of difference between controls and treated animals 1 vs 1 d not significant control vs 2 d not significant control vs 3 d <0 001, control vs 5 d <0 001 control vs 6 d <0 001 (b) Gentamicin treatment for 3 and 5 days respectively The animals were killed 21 days after the last injection P values for the significance of the difference between controls and treated animals control vs 3 d <0 001 control vs 5 d <0 001

RESULTS

Cytochemical findings

The sensory cells from the hearing organ of control lizards contain about 19 pg RNA/cell The intercellular variation is minimal as also is the variation between animals (Fig 1) This table also demonstrates the effect of gentamicin on the sensory cells Even a single dose of gentamicin induces a small reduction in the RNA content of the cell types studied After treatment for 3 days there is a highly significant decrease of the RNA content of greater than 30% below the control level Further gentamicin injections result in an almost 50% depletion of the RNA content of the cells Three or 5 days daily administration of gentamicin and a free period for 21 days did not allow a restoration of the reduced RNA content, and the values remained at almost exactly the same low levels as when the animals were sacrificed the day after treatment for 3 or 5 days respectively

Fig 2 shows the RNA values in the acoustic ganglionic cells The control cells contain around 45 pg RNA, with very small intercellular variations After injection of gentamicin, the cellular RNA content decreases linear in relation to the number of injections After two injections there is a significant decrease in the amount of ganglionic cell RNA Animals that received six injections and were sacrificed the day after the last injection retained only about 50% of the original content of RNA When gentamicin was administered for 3 days and the lizards were killed 21 days later, the content of RNA in the ganglionic cells was almost restored and not significantly different from control levels of RNA However, when the drug was given for 5 days and



... from the ... mina ... is the ... ations ... 3 5 ... and 6 days respectively The animals ... the day after the last injection P values for the significance of the difference between controls and treated animals control vs 1 d not significant control vs 2 d <0 02 control ... injection P values for the significance of the difference between controls and treated animals control vs 3 d, not significant control vs 5 d <0 005

After

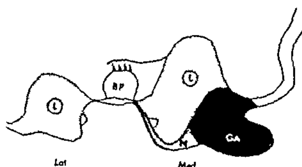


Fig 3 Schematic drawing of a cross section through the basilar papilla and surrounding structures The basilar

the limbic tissue

the animals killed 21 days later, the low amount of RNA persisted in the ganglionic cells

Morphological findings

Normal anatomy The basilar papilla is a club-shaped organ with a length of about 425 μm The organ is situated in a kidney-shaped opening in the surrounding supporting tissue, also called the limbic tissue (Fig 3) This tissue supports the sensory epithelia located in this part of the labyrinth, i e the macula lagena, the basilar papilla and the macula sacculi The ilar papilla harbours sensory and support g cells and it rides on a basilar membrane retched over the opening in the limbus (Fig 4) The sensory cells are cylindrical and have a length of 25–45 μm (Fig 5) They consist of two populations one ventral (apical) and one dorsal (basal) The ventral population is covered by a tectorial membrane Processes from the supporting cells interpose between the sensory cells, thus isolating these from each other Nerve fibres enter from the medial aspect and terminate with synapses on the sensory cells Each sensory cell harbours a sensory hair bundle inserted in the cuticular plate that forms most of the free surface exposed to the endolymph (Fig 4) For further information on general anatomy see Bagger Sjöbäck & Wersäll (1973), Bagger Sjöbäck (1976) The acoustic ganglion consists of a rounded population of ganglionic cells located in the limbic tissue medial of the basilar papilla (Fig 3) The ultrastructure of the ganglionic cells has not been studied, either in normal or pathological conditions

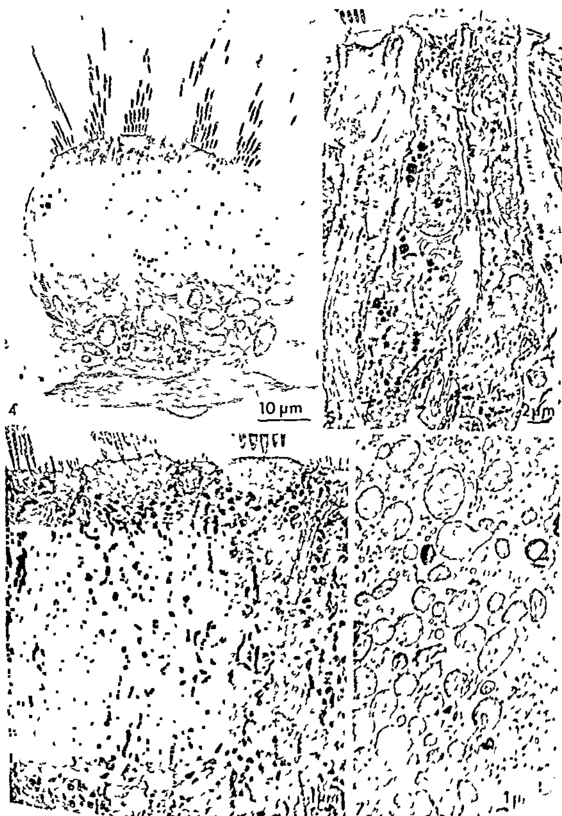
Pathological anatomy Ultrastructural studies on gentamicin treated animals show a large variation in the response to the drug Early signs of morphological degeneration however, may be seen in animals that have received the drug for 7 days The gross appearance of the basilar papilla in these animals is normal The sensory hairs are intact and the cellular outlines are unchanged (Fig 6) Large variations occur in the way individual cells react to the drug Many sensory cells appear totally normal with no distinct signs of changes, while others can show incipient swelling and vacuolization of the cytoplasm In these cells, signs of incipient degeneration of the mitochondria may also be seen (Fig 7) To summarize the findings, one can conclude that in the first test group of animals no distinct pattern of degeneration can be established which is valid for all cells in the same specimen The animals of the second test group that were given the drug for 3 or 5 days and sacrificed 21 days after the last injection present marked signs of morphological change The sensory cells are distorted and irregular in shape (Fig 8) The sensory hairs however, have a normal appearance without signs of hair fusion, etc In Fig 10, the nucleus is seen disintegrating with clumping of the chromatin material The cytoplasm contains

Fig 4 Electron micrograph showing a cross-section through the dorsal part of the basilar papilla in an untreated animal Note the tall bidirectionally oriented sensory hair bundles

Fig 5 Normal sensory cells in an untreated animal

Fig 6 Sensory cells with early signs of morphological changes The cytoplasm and nucleus however appear quite normal Occasional changed mitochondria may be seen Gentamicin treatment 7 days

Fig 7 Slightly swollen mitochondria and occasional myelin figures in a sensory cell Gentamicin treatment 7 days


4
10 µm
2 µm
1 µ


8

several vacuoles and in some cells displays an abnormally strong stainability The mitochondria are in various stages of degeneration with swelling changes in the structure of the cristae and myelin figure formation Aggregates of disintegrating cellular components and debris are seen contained within autophagocytotic bags" (Fig 9) Consequently the animals in this test group present marked signs of cellular change significantly stronger than those of the first test group The supporting cells show no morphological signs of degeneration in any of the animals treated with gentamicin As stated earlier, the pathological morphology of the ganglionic cells was not investigated

DISCUSSION

The purpose of the present study was to define a biochemical background to the morphological degeneration observed in sensory cells of the hearing organ after administration of ototoxic antibiotics As a biochemical parameter, determination of cellular RNA content was chosen There are several advantages with such determinations, for example (*a*) they can be performed on a single cell level with great accuracy (Edstrom, 1964), (*b*) RNA is known to be a sensitive indicator of the functional state in nerve cells (Edström, 1964 Hyden, 1960, Jarlstedt, 1966), (*c*) RNA is a vital substance of ultimate importance for protein synthesis and has been suggested to be an important substance in sensory cell function (Ruben 1969) The results show that even after 3 days of treatment with gentamicin, the RNA content is significantly reduced to 65% of the original value A treatment period of 5 days reduces the cellular RNA content to 50% of the control level At the time when a definite decrease in the amount of RNA becomes evident, there are as yet no constantly detectable ultrastructural changes This underlines the statement that biochemical changes are the forerunners of morphological changes It is therefore suggested that the lack of morphologically visible degeneration cannot be taken as an indication of preserved cellular stability, since there may be a natural time lag between biochemical effects and the onset of morphological changes This statement is supported by the findings in the second test group where 21 days after the point in time when the RNA content in sensory cells was significantly reduced by gentamicin administration, the sensory cells also showed massive cellular derangement even when no gentamicin was administered during this free period At this time there is a good correlation between the previously observed and persistent biochemical findings and the morphological state, since the low RNA values persist The aminoglycoside antibiotics have been regarded as relatively incapable of penetrating into the cell (Andre, 1956, Robson & Sullivan 1963) Possibly these antibiotics act primarily on the plasma membrane, creating a secondary leakage of the drug into the cell as postulated by Bagger Sjoback & Wersall (1976) Once within the cell, the drug affects the ribonucleic acids as demonstrated in this study The mode of action of aminoglycoside antibiotics upon eukaryotic cell RNA is not known at present In microorganisms these antibiotics impair certain ribosomal functions and thus disturb normal protein synthesis (Pestka, 1971) Possibly this mode of action can be applied to cells of the inner ear, although this has not yet been fully elucidated The basal system for protein synthesis, geno fun

Fig 8 Severe changes in sensory cells from an animal in the second test group Vacuolization of the cytoplasm and derangement of the nuclei is evident Aggregates of degenerated mitochondria and autophagocytotic bags can also be seen The sensory [illegible]

Fi[illegible] [illegible] Gentamicin treatment 3 days with 21-day free period

Fig 10 Sensory cells from an animal of the second test group Note the differing staining properties of the three cells Nuclear changes and aggregates of deranging organelles are present in all the cells Gentamicin treatment 5 days with 21-day free period

tion, i e DNA dependent RNA synthesis, coupling of messenger RNA and ribosomes to form polysomes, function of transfer RNA and subsequent polypeptide chain formation, is essentially the same in bacteria and eukaryotic cells The influence on cellular RNA by the drug can be accomplished in several ways (1) Blockage of DNA-dependent RNA synthesis comparable to the well known action of another antibiotic, actinomycin D (2) Blockage of protein synthesis in the manner described for puromycin—cessation of protein synthesis results in lack of enzymes necessary for RNA synthesis, thus finally resulting in diminished cellular RNA content (3) Induction of reduced RNA stability and hence accelerated loss of RNA These are some of the tentative explanations of the severe loss of RNA The degree of diminished RNA content is such that an impaired protein synthesis must be presumed, which in turn can account for observed morphological changes

It is interesting to note that the acoustic ganglionic cells are affected in a manner similar to the effect on sensory cells Even after two injections of gentamicin there is a significant decrease in the RNA content and after six injections the amount is reduced to 50% of original level This finding compares well at of Kellerhals et al (1968) who ob- ved degeneration of spiral ganglionic cells er intra aural injections of kanamycin in guinea pigs Floberg et al (1949) described loss of RNA in vestibular ganglion cells after streptomycin treatment and interpreted this as an inhibition of nucleic acid production in these cells Whether streptomycin and kanamycin exert a direct toxic action on ganglionic cells as stated by Floberg et al (1949) and by Kellerhals et al (1968) or whether the decrease is related to secondary changes due to deteriorated function of the sensory cells can not be definitely established through this study However, the time course for and degree of the RNA changes are almost identical in the two cell types thus favouring the concept of a direct action

In the present material there was one important difference between sensory cells and ganglionic cells in the biochemical response to gentamicin The animals which received three injections of the drug and then were killed 21 days later had restored their RNA content which was not the case with the sensory cells. When, however, the drug was administered for 5 days and the animals sacrificed 21 days later, there had been a progressive loss of RNA and these ganglionic cells had significantly lower amounts than the cells from animals of the first test group which received five injections and were killed the day after the last injection

As measured by the parameter used in this study, a moderate toxic action is biochemically irreversible but when the action has progressed beyond a certain limit the damage is reversible and cell death is imminent In functional terms the reversibility is perhaps of less significance, since the degeneration of hair cells is irreversible

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ZUSAMMENFASSUNG

Zu der Eidechse *Calotes versicolor* sind die Wirkungen auf den RNS-Gehalt isolierter Ganglien und Sinneszellen des Innenohres nach einer kurzzeitigen Zugabe von Gentamicin untersucht worden Wirkungen dieser Droge auf biochemische Parameter werden ultrastrukturellen Veranderungen in den Sinneszellen desselben Materials gegenubergestellt Nach einer täglichen intraperitonealen Injektion (Dauer 3 bis 6 Tage) verringert sich der RNS Gehalt in Sinnes und Ganglienzellen um 30–50% Zu diesem Zeitpunkt wurden keine konstanten ultrastrukturellen Veranderungen festgestellt In einer zweiten Ver ... 3 oder 5 Genta

... Bei den mit 3 Injektionen ...

... rehalt

... nner

... ruppe

bestimmte morphologische Veranderungen beobachtet Die Beziehungen zwischen zytochemischen und morphologischen Befunden sowie mögliche Wirkungsmechanismen von Gentamicin auf den RNS-Gehalt der Innenohrzellen werden diskutiert

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XYLENE EXPOSURE

Electronystagmographic and Gaschromatographic Studies in Rabbits

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Abstract Complaints of vertigo from people who are exposed to industrial solvents have focused interest on their toxic effect on the nervous system In order to evaluate the influence of an organic solvent xylene on the mammalian vestibular system a series of rabbit experiments were performed To achieve a constant concentration the xylene was infused as a lipid emulsion Blood concentrations were estimated by gas chromatography Electronystagmography in darkness revealed that at blood [illegible]

the alcohol and the xylene reaction was that rotatory nystagmus responses were exaggerated The relations between the present findings and the reactions and blood concentrations in people exposed to industrial solvents discussed

use of paints and industrial solvents containing aliphatic and aromatic hydrocarbons has become widespread The influence of these solvents on the mammalian organism has thus become a target for intense interest in occupational medicine (Åstrand 1975, Åstrand et al 1972) It is well known that symptoms from the central nervous system such as headache, nausea fatigue and vertigo may occur upon the inhalation of several organic solvents (Browning 1965 Goldie 1960) These symptoms are often very vague and it is difficult to find specific objective signs at the clinical examination The need for further research utilizing neurophysiological methods is evident (Seppalainen, 1975) Certainly, this will facilitate the determining of accurate safety limits in industrial work and the detection of early symptoms of intoxication

Many intoxication symptoms indicate an action on the central part of the vestibular system Alcohol intoxication is a well known instance, it gives subjective symptoms rather similar to those described for industrial solvents In a series of papers, Aschan et al (1956, 1957, 1964*a*, 1975) showed that alcohol intoxication gave a typical pattern of positional nystagmus (PAN) The beat direction and intensity of PAN could be predicted if the blood alcohol concentration was followed simultaneously Electronystagmographic recordings should offer a reliable method for studying the effect of solvents on this specific part of the central nervous system The aim of the present investigation was to evaluate this possibility in animal experiments The relation between the action on the vestibular system and the blood concentration of the solvent will be investigated Xylene, which was chosen as a model substance in the present investigation, is widely used in the paint printing, rubber, and plastic industries It is also present in the mixture "thinner , for common household use but also abused by some youngsters for inebriation (cf sniffing glue)

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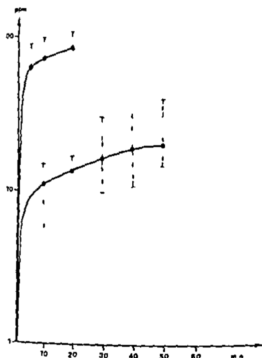


Fig 1 Mean arterial blood concentration and range expressed in mg per kg of blood (ppm) during intravenous infusion of xylene (dissolved in Intralipid) The upper curve represents 8 experiments with an infusion rate of 12 0 mg of xylene per minute and the lower curve 10 experiments with an infusion rate of 2 2 mg of xylene per minute

METHODS

Rabbits weighing 2–4 kg were mounted in a box which could be darkened by closing the lid A conventional head holder secured a steady position when the box was turned laterally Subcutaneous needle electrodes were inserted for electronystagmographic recordings (Aschan et al, 1964*b*, Aschan, 1970) The animal box was mounted on a Stille rotating platform which was designed for rotatory testing During the experiments the animals were repeatedly tested for spontaneous and positional nystagmus in left and right lateral positions All animals were free from spontaneous or positional nystagmus before intoxication The nystagmographic response to clockwise and anti clockwise rotation was also tested using acceleration of 5°/sec² up to a speed of 75°/sec After 60 seconds of constant speed an analogous deceleration took place

In 15 preliminary experiments, xylene (commercial grade m xylene with less than 2% of o-xylene) was administrated by inhalation using an adjustable evaporator and a semi open system In 24 experiments xylene was dissolved in Intralipid® (an emulsion of lipids, used for human parenteral nutrition prepared by Vitrum AB, Stockholm) and administered by continuous intravenous infusion A 10% solution of xylene was prepared immediately before each experiment An ear vein was cannulated and a polyethylene catheter was introduced at least 15 cm in order to reach the central veins with a rapid blood flow The polyethylene catheter was connected to an infusion pump which was placed on the rotating platform and supplied with a sliding elec

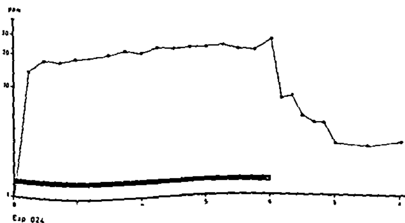


Fig 2 Arterial blood concentration in a typical ment during and venous infu The bar infusion

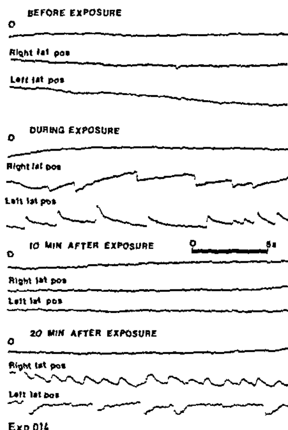


Fig 3 Electronystagmographic recordings from a rabbit before during and after xylene exposure Recordings were performed in upright right lateral and left lateral positions Upwards in the graph indicates an eye ovement to the right

l connection For collecting blood sam s a polyethylene tube was introduced in an ear artery on the opposite side

For the analysis of xylene, 0 5 ml of blood was withdrawn with a syringe rinsed in heparin The blood was immediately transferred to a tube with 0 5 ml of a solution containing 10% v/v of iso propanol and 0 0004% (v/v) of toluence (used as internal standard) in water The added amount of blood was weighed Usually within 2 hours 0 1 ml of the mixture was transferred to a 1 ml glass vial which was sealed hermetically with a silicone coated rubber membrane The vial was shaken vigorously for 10 sec and was then stored at room temperature for at least 30 min (but not more than 2 hours) With an airtight syringe equipped with a fine needle, 0 1–0 2 ml of the gas phase was aspirated and injected into a Varian 1400 gas chromatograph This was equipped with a 5% OV-17 glass column (1 5 m×2 mm I D) and a flame ionization detector The column temperature was 50°C and the detector temperature was 200°C For each experiment a calibration curve was constructed This was done by aspirating a few ml of blood from the arterial catheter before the infusion of xylene was started Half a ml of the blood was added to each of several test tubes containing the isopropanol–toluene–water solution and various amounts of xylene The calculations were based on the peak height ratio xylene/toluence

Using the head space method, symmetrical peaks of xylene and the internal standard were obtained The retention time for toluene was about 1 min and for xylene about 2 min The calibration curve was linear in the range 1–100 ppm (mg of xylene/kg of blood) The reproducibility at various levels was tested by several analyses from the same sample of blood to which a known amount of xylene had been added SD_{rel} was found to be 3%

RESULTS

When xylene was administered by inhalation the blood level of the solvent was unpredictable and very rapid fluctuation occurred from minute to minute, even though an evaporator giving a uniform gas supply was used

When administering xylene intravenously a good correlation was achieved between the

Table I *Arterial concentration of xylene and incidence of PXN I at 10 minutes after starting xylene intravenous infusion (2 2 to 12 0 mg of xylene per minute)*

Number of expts *n*	Arter conc of xylene (ppm)	Incidence of *PXN I*
6	<10	0
11	10–30	2
7	>30	7

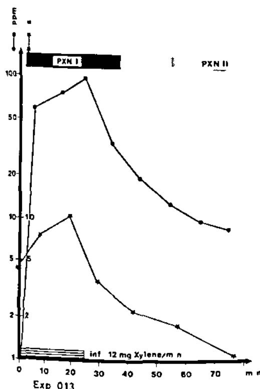


Fig 4 Xylene arterial blood concentration (●—●) and duration of postrotatory nystagmus (×—×) during and after intravenous infusion of xylene The presence of PXN I and PXN II is indicated by bars

blood level and the infusion rate The blood levels obtained in 18 experiments with different rates of infusion are presented in Fig 1 A very rapid rise of the blood level during the first 10–20 min of infusion was followed by a slower rise to a steady state level With an infusion rate of 2 15 mg xylene/min a steady state level was reached after 60–100 min (Fig 2) The elimination of the solvent after cessation of the infusion was very rapid during some 10 min, but it then continued at a slower rate (Fig 2)

Electronystagmographic recording

During inhalation of xylene most of the rabbits showed a positional nystagmus, left beating in the right lateral position and right beating in left lateral position named further on as positional xylene nystagmus (PXN I Fig 3) In some cases the xylene blood level was above 100 ppm Many of these rabbits showed respiratory distress and some died

In 24 experiments, xylene was given by intravenous infusion The incidence of positional nystagmus and the related blood level of xylene at 10 min after starting the infusion is presented in Table I With a blood level of 30 ppm or more, all the rabbits showed PXN I When a steady state level higher than 10 ppm was avoided, none of the rabbits got nystagmus, even when the infusion was continued for more than 4 hours In 6 cases, after cessation of the infusion, there appeared a faint positional nystagmus, left beating in left lateral position and right beating in right lateral position (PXN II) Five of these rabbits *belonged to the high xylene concentration* group (Table I) The duration of this second phase varied greatly, from 20 min to several hours (Figs 3 and 4)

The rotatory tests in the xylene experiments showed that during the PXN I period the rotatory nystagmus was greatly exaggerated, both the speed of the slow component and the beat amplitude being higher The post acceleration nystagmus had a considerably longer duration (Figs 4 and 5)

DISCUSSION

Our experiments confirm the suggestion that xylene affects the vestibular system (Larsby et al , 1976) The positional nystagmus that occurs during administration of xylene has not to our knowledge, been described previously

During the first phase of the alcohol nystagmus the direction of the beats is to the right in the right lateral position and vice versa (PAN II) From earlier experiments in man and rabbit (Aschan et al , 1956, 1964*a*, Aschan 1958) it was concluded that the mechanism that elicits PAN affects the central vestibular pathways but demands intact labyrinthine function Bergstedt (1961) assum the maculae peripherally are esse elicitation of PAN Another t

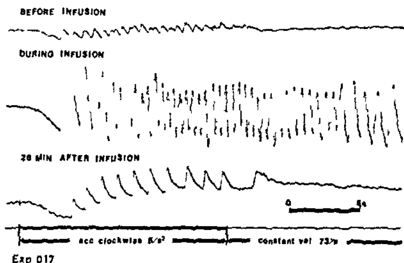


Fig 5 Nystagmus responses to acceleration before during and after xylene exposure The acceleration period and the subsequent constant speed rotation are indicated below

Myles, 1974, Money et al , 1974) is that PAN I is elicited by the alcohol, with a low specific weight, entering the cupula making it lighter and so giving it a higher buoyancy in the surrounding endolymph When the alcohol later reaches the endolymph, giving it a lower specific weight, the relative buoyancy of the cupula is diminished The cupula moves in the opposite direction, eliciting positional alcohol nystagmus with a reversed beat direction (PAN II) Money & Myles (1974) have given eavy water, deuterium, to rabbits and hu and found a positional nystagmus with a direction similar to PAN II They explain by the same discussion concerning the changes in specific weight of the cupula and later the endolymph As deuterium is not believed to have any pharmacological effects on the nervous system the theory seems attractive

If this theory of peripheral action is correct, even for xylene, with its specific weight of 0 86, PXN I should have the same beat direction as PAN I Yet this is not the case Obviously PXN is elicited in the central vestibular system by some mechanism other than PAN The rotatory tests performed also indicate varying toxic effects in the central vestibular system In the rabbit, alcohol intoxication reduced or temporarily completely inhibited the rotatory induced nystagmus, whereas xylene exaggerated the same nystagmus responses It would be rather difficult to explain these observations as being elicited in the peripheral labyrinthine organ The change in the direction of the xylene nystagmus from PXN I to PXN II could be explained by a central rebound mechanism

The difficulty and importance of getting a uniform level of the solvent in the blood in this kind of animal experiments was demonstrated in our trial by administering the substance by inhalation No definite conclusions could be drawn from these experiments For that reason a new method for the administration of xylene was created Administering the substance by intravenous infusion seems to give predictable and constant levels in the blood It will be preferred in subsequent studies, which are designed to investigate neurophysiologically the effect of xylene in the central nervous system However, even with this method of administration, occasional fluctuations in the ventilation influence the levels of xylene in blood, as most of the solvent is probably eliminated by the lungs

Our experiments demonstrate a close correlation between the arterial blood level of xylene and the effect on the vestibular system A rapid equilibration between xylene in the

blood and in the brain tissue is indicated This is also supported by human experimental studies of the pharmacokinetics of toluene, carried out by Sato et al (1974), who applied a three compartment model, one of which was composed of blood and vessel rich organs such as the brain

The most interesting observation is that xylene an aromatic hydrocarbon substance, gives a well defined positional nystagmus pattern depending upon the actual blood xylene concentrations The parallel between objective nystagmus findings and blood concentration when testing with alcohol, as well as xylene, proves the validity of the working hypothesis when commencing this type of research On the other hand the opposite beat directions of PAN and PXN indicate different central activating mechanisms

All rabbits show nystagmus at a blood xylene concentration above 30 ppm At lower xylene concentrations the incidence of PXN diminished but in two experiments concentrations of 10 and 14 ppm were enough to elicit a positional nystagmus According to Swedish law, the highest permitted air content of xylene in industrial work is 100 ppm The relationship between the concentration of toluene in inspiratory air and in blood has been studied under various conditions by Åstrand et al (1974) During hard work in air contaminated with 100 ppm of toluene the arterial concentration was about 2 7 mg/kg (ppm) As the partition coefficient of xylene between blood and air is about twice as high as that of toluene (Åstrand, 1972), work in an environment containing 100 ppm xylene may result in an arterial concentration above 2 7 ppm presumably 2×2 7 The blood alcohol concentration that elicits PAN is 0 02% for man and 0 2% for rabbit (Aschan et al , 1956, 1957, 1964*a*, *b*) Hence it is reasonable to assume that vestibular symptoms in man appear at blood xylene concentrations approximately 10 times lower than those giving nystagmus in our rabbit experiments Thus the person who works in an environment with a xylene concentration close to the permitted limit may get a blood xylene concentration necessary for vestibular symptoms

ACKNOWLEDGEMENT

The authors wish to thank laboratory engineer Håkan Barreng for technical assistance and engineer Bjorn Norlander for valuable advice concerning the gas-chromatographic methods

ZUSAMMENFASSUNG

Da Personen die industriellen Losungsmitteln ausgesetzt sind uber Schwindel klagen wurde die besondere Aufmerksamkeit auf die eventuelle giftige Wirkung dieser Losungsmittel auf das Nervensystem gelenkt Um den [illegible]

Xylene im Blut einen Lagenystagmus hatten Die Schlagrichtung war entgegengesetzt der Richtung bei Alkohollagennystagmus Außerdem war im Gegensatz zu dem bei [illegible] verstarkt Der Z sam [illegible]

wird diskutiert

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BONE-CONDUCTED STIMULATION IN ELECTROCOCHLEOGRAPHY

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(Received October 1, 1976)

Abstract The mechanical vibration patterns close to the [illegible]

superior to the clicks with regard to the vibration spectrum At 500 Hz a considerable distortion was observed in the accelerometer signal also when using tone bursts This distortion was presumably due to resonant vibrations in the skull itself and may be a source of error not only when using stimuli of short duration as in [illegible]

soft tissues covered the point of application This could be of advantage in bone-conduction ECoG performed at ear surgery

Electrocochleography (ECoG) is by now an established electrophysiological aid in the clinical evaluation of the peripheral auditory function (Cullen et al 1972, Yoshie 1973, Eggermont et al , 1974, Beagley et al 1974, Simmons, 1975)

Various systems for electrode placement have been suggested One of these is based on a needle electrode, which perforates the eardrum and the tip of which is placed on the promontory in the middle ear The reference electrode is usually placed on the ear lobe or the mastoid This electrode configuration mainly picks up the compound action potential (AP) of the auditory nerve which occurs in the latency range 1 5–5 msec after stimulus onset In order to produce as clear a response as possible the rise time of the acoustic stimulus must be kept short

One advantage of the ECoG method is that masking of the non test ear is not required This is because electrical activity generated on the contralateral side is of negligible amplitude at the test ear electrode, compared with the ipsilaterally generated activity

In conventional pure tone audiometry, masking is often a problem when measuring bone-conduction thresholds This is particularly true on patients with hearing impairment which involves both conductive and sensorineural components ECoG with bone-conducted stimulation could thus be a potentially valuable method in such cases, particularly in the preoperative evaluation of the cochlear function in candidates for middle ear surgery

However, most reports published on ECoG have employed air-conducted stimulation Aran (1973) used a vibrator of type Bruel & Kjaer 4810 Mini Shaker for bone conducted stimuli, applied on the patient s forehead Yoshie (1973) applied bone conducted stimuli to the subject's forehead by means of a regular bone vibrator He found that when short tone pips at 4 kHz were used there was a good correlation between ECoG responses obtained to air and bone-conducted stimuli However, using clicks instead of tone pips, he c

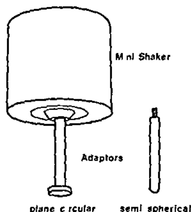


Fig 1 Mini Shaker with the two adaptors used in the study

a difference between the AP response wave forms to air and bone conducted stimulation This difference was assumed to be related to the differing spectral distribution of acoustic energy between the air and the bone conducted clicks

Currently available bone vibrators used for audiometers and hearing aids are electro mechanical transducers with rather poor characteristics, particularly as regards frequency response linearity and distortion (Dirks & Kamm, 1975) Such vibrators may therefore be suspected of having a rather limited ability to reproduce the transient uli used in ECoG such as wide band s filtered clicks or short tone bursts hermore the human skull constitutes a ery complex mechanical structure the vibration patterns of which in response to transient bone conducted stimuli are not easily predictable (Kirikae 1959)

It was therefore decided to study the ability of various vibrators to transfer signals of different types faithfully into mechanical vibrations of the human skull The aim was also to evaluate the influence of different vibrator locations on the skull and different static forces of application

METHOD

The vibrations transferred to the cranium from the bone vibrators were investigated on the intact skulls of three human cadavers During the experiments the cadaver was placed supine on a stiff laboratory cot which also supported the head A miniature accelerometer (Bruel & Kjaer type 8303 weight 3 5 g) was used as a vibration pick-up One end of a brass rod (length 35 mm diam 1.8 mm, weight 1 g) was screwed onto the accelerometer A small hole was drilled in the promontory in the middle ear In this hole the other end of the rod was placed and firmly fixed to the bone tissue by means of *cranial cement The signal from the accelerometer* was amplified (Bruel & Kjaer 2603) and fed to an oscilloscope (Tektronix 561A) The accelerometer signal was displayed on one of the two oscilloscope channels and the electrical input to the vibrator displayed on the other channel A Tektronix C12 (Polaroid) oscilloscope camera was employed for photography The photographs were later evaluated with regard to how the electrical signal driving the vibrator was converted to a mechanical signal in the skull as shown by the accelerometer output

Two types of vibrator were used in the study One was an ordinary audiometer vibrator, Radioear B70A with a standard elastic headband the other a Bruel & Kjaer type 4810 Mini Shaker On the Mini Shaker various adaptors could be screwed which made contact with the skull and transferred the vibrations from the Mini Shaker (Fig 1) One adaptor tested was a 6 mm diameter aluminium rod with a plane circular tip surface of approx 1 75 cm² The other adaptor used in the study was a plexiglass rod of 6 mm diameter with a nearly hemispherical tip Both adaptors had a length of approximately 60 mm

A special holder was manufactured for the Mini Shaker, by means of which the vibrator s position and direction could be adjusted and various static application forces obtained Static forces from 3 to 10 N were studied These forces were determined by calibration on an ordinary laboratory scale

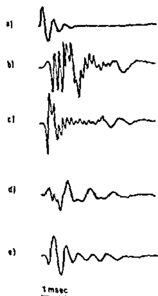


Fig 2 Filtered clicks of 2 kHz carrier frequency (*a*) Electrical input to vibrator (*b*) Accelerometer output with B70A on forehead (*c*) do on mastoid (*d*) Accelerometer output with Mini Shaker on forehead (*e*) do on mastoid with exposed bone surface

The vibrators were placed either on the mastoid process, ipsilateral to the accelerometer, or in the centre of the forehead In addition the Mini Shaker was tested with its vibrations transferred direct to the bone surface of the mastoid process, i e with the soft tissues that normally cover the bone, removed

The electrical signals used to drive the vibrators were of two types commonly used in ECoG One was filtered clicks, generated by feeding short, rectangular pulses to band pass filters The filtered clicks had centre frequencies of 1, 2, 4, and 8 kHz and a time constant of approx 0 5 msec of the exponentially decaying amplitude The other type of stimulus consisted of short tone bursts The tone frequencies used were 0 5 1 2, 4 and 8 kHz Rise and fall times equalled one period of the tone frequency, and the plateau duration was either six periods or 4 msec, whichever was the longer

In addition to the study on the vibrations in human skulls, a mechanical coupler (artificial mastoid, Bruel & Kjaer 4930) was also used and the output signal from its built in accelerometer evaluated

RESULTS

Filtered clicks

Using the B70A vibrator, the output signal from the accelerometer was found to be grossly distorted as compared with the electrical input to the vibrator The spectrum of the accelerometer signal showed little relation to the carrier frequency of the clicks (1 2 4, or 8 kHz), the signal was dominated by a periodic activity in the 5 kHz region No difference in this respect was seen with regard to vibrator location (forehead or mastoid)

When the Mini Shaker was used the qualitative agreement between accelerometer output and the input signal to the vibrator improved only slightly Generally speaking when the soft tissues covering the mastoid process were removed and the vibrator adaptor applied to the cranical bone direct

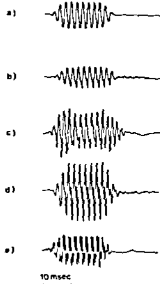


Fig 3 Tone bursts at 500 Hz Rise and fall times increased to four periods (*a*) Electrical input to vibrator (*b*) Accelerometer output with B70A on forehead (*c*) do on mastoid (*d*) Accelerometer output with Mini Shaker on forehead (*e*) do on mastoid with exposed bone surface

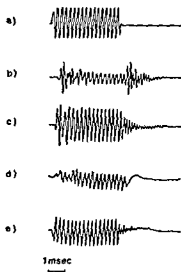


Fig 4 Tone bursts at 4 kHz (*a*) Electrical input to vibrator (*b*) Accelerometer output with B70A on forehead, (*c*) do on mastoid (*d*) Accelerometer output with Mini Shaker on forehead (*e*) do on mastoid with soft tissues

the accelerometer signal resembled the electrical input better than when the vibrator was placed onto the intact skin

Fig 2 illustrates some of the wave forms recorded when using filtered clicks of 2 kHz centre frequency

When the vibrator was placed on the artificial mastoid and filtered clicks were used e output signal obtained from its acceler- ter also showed a highly distorted wave-

Tone bursts

When short electrical tone bursts were fed into the Radioear B70A vibrator, the output signal of the accelerometer provided a reasonably good copy of the vibrator input signal This was particularly true when the vibrator was placed on the mastoid process, except at 500 Hz when the accelerometer signal showed a considerable harmonic distortion (Fig 3*b*, *c*) When the vibrator was placed on the forehead a rather pronounced on–off effect was noted at 4 and 8 kHz, i e the accelerometer produced a larger signal immediately after the onset and the offset of the tone bursts than during the actual duration of the bursts This on–off effect was dominated by a frequency of approx 6 5 kHz for both burst frequencies, and it tended to decrease when the rise and fall times were increased from one to two periods At most signal level during these on–off events rose approximately 10 dB above the level recorded in the steady state

In one skull, the ear under study was subjected to drilling to an extent representative of a combined approach tympanoplasty (CAT) After the drilling was completed the accelerometer signals were re recorded using the forehead placement of the B70A vibrator No significant difference was seen in accelerometer signals, signal levels were the same *within a 4 dB range Thus the bone* tissue removed by the drilling had no significant influence on the recorded vibration levels

When the Mini Shaker was used, the wave-form of the accelerometer output generally conformed well with the vibrator input signal for burst frequencies 1–8 kHz This was the case when using the adaptor with a plane circular tip as well as the one with a semi-spherical tip, and for both forehead and mastoid placements No qualitative difference was observed with the vibrator on the mastoid process covered by soft tissues as compared with when the bone was exposed to the adaptor

Table I *Mean values (over burst frequencies 1–8 kHz) of vibration burst levels recorded at different forces of application (Mini Shaker, plexiglass adaptor with hemi-spherical tip)*

Levels obtained at 5 N are used as reference Three vibrator positions were used forehead mastoid with soft tissues, and mastoid with exposed bone surface

	Application force (N)			
	3	5	7 5	10
Forehead	−4 0 dB	0	2 7	5 5
Mastoid soft tiss	−1 5	0	−1 4	−1 5
Mastoid bone	1 0	0	0 2	0 1

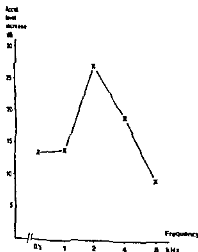


Fig 5 Average increase in vibration levels measured on two skulls at different burst frequencies when Mini Shaker was applied in direct contact with the mastoid bone surface as compared to when soft tissues covered the mastoid

However, when 500 Hz bursts were used a rather pronounced harmonic distortion was obtained in the accelerometer signal (Fig *3d e*) This distortion was dominated by the second harmonic when the vibrator was applied against the skin, on the forehead, or the mastoid However, when using the hemispherical adaptor, placed direct on the bone of the mastoid process, the third harmonic was most apparent

In the skull, where drilling corresponding to a CAT was performed, the accelerometer signals were re recorded after the drilling, using the Mini Shaker with the hemispherical tip adaptor against the exposed bony mastoid The levels obtained were on the average 2 6 dB lower (range −4 9 to +0 5 dB) than those obtained before the drilling

Fig 4 shows some typical samples of accelerometer signals obtained when using 4 kHz tone bursts

A comparison of accelerometer signal levels obtained when using the Mini Shaker and the B70A vibrator, placed on the mastoid and fed with equal electrical input levels showed frequency-dependent differences which agreed within ±5 dB with the differences in electrical input levels to the two vibrators that corresponded to psychoacoustic thresholds The latter were determined on 9 normal hearing young subjects

When the vibrators were placed on the artificial mastoid, its built in accelerometer produced signals whose wave forms reproduced reasonably well those of the electrical input to the vibrators Some ringing particularly evident after the end of the tone bursts, was noted, though, of a form different from that occasionally observed when experimenting on human skulls

Influence of application force

When using the Mini Shaker fed with tone bursts, the accelerometer signals obtained using application forces of 3, 5, 7 5, and 10 N showed a consistent relation to application force only when the vibrator was placed on the forehead Table I shows the mean values (over burst frequencies 1–8 kHz) of accelerometer signal levels obtained, referred to those at 5 N application force, for three different vibrator placements Levels recorded at 500 Hz were omitted because of the high degree of distortion present With the Mini Shaker placed on the mastoid no significant influence of application force was found on signal levels recorded This was true of the vibrator placed directly against the cranial bone as well as against the soft tissues normally covering the mastoid When the vibrator was placed on the forehead the signal levels increased with increasing force of application The relation was tested with linear regression analysis (least squares method) and the correlation coefficient was found to be significantly greater than zero ($p<0\ 1\%$)

Influence of soft tissues on vibration transmission

The influence of soft tissues (skin, fat, muscle) on the transmission of vibrations from the vibrator to the cranial bone was

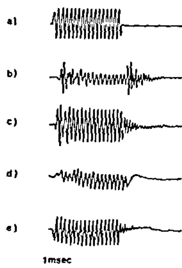


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	Appl cation force (N)			
	3	5	7.5	10
Forehead	−4 0 dB	0	2 7	5.5
Mastoid soft tiss	−1.5	0	−1 4	−1.5
Mastoid bone	1 0	0	0.2	0 1

study an on–off effect was occasionally observed in the accelerometer response to tone bursts, particularly at higher frequencies. Most likely, this was caused by the skull being brought into a transient state of vibration at some resonance frequency by the steep onsets and offsets of these bursts.

The rather inconsistent dependence of recorded vibration levels on force of application agrees with the conclusion of Dirks & Kamm (1975). With the Mini Shaker on the forehead—the only position where a significant influence was found—the increase of accelerometer signal level with force of application was nearly linear, with a slope of approximately 1.3 dB/N.

The experiments performed on the artificial mastoid showed that, at best, it can be used for a very coarse estimate of the vibrations produced. For example, the pronounced distortion present when recording from the human skull at 500 Hz burst frequency was entirely absent in the signal from the artificial mastoid. This was not unexpected, since the purpose of the artificial mastoid is to imitate the mechanical impedance of the skull surface and there is no further similarity to the mechanical structure of the human skull. An evaluation of how the electrical signal, fed into a vibrator, is converted into a mechanical signal by the properly loaded vibrator can be made, but how the skull responds to this mechanical signal is a completely different matter.

With regard to the two vibrator types studied, the Mini Shaker seems to be somewhat better than the Radioear B70A, considering the vibration patterns observed. It has the additional advantage of a greater power handling capacity and less non-linear distortion. On the other hand, it weighs considerable more, is of larger dimensions, and requires some special arrangements for holding and producing the required force of application.

The increase of 10–25 dB in vibration levels noted when the Mini Shaker adaptor was placed direct on the exposed bone of the mastoid process, as compared with being placed on intact soft tissues, may be of possible use in some situations. One such situation occurs when ECoG with bone-conducted stimuli is performed in connection with ear surgery, the operation field giving the surgeon free access to the surface of the mastoid bone. Furthermore, it should be quite possible to perform ECoG with bone conducted stimuli under local anesthesia. A small retroauricular skin incision and the drilling of a small cavity in the bone of the mastoid could be done without any greater discomfort to the patient.

Apart from the increased signal levels available, another advantage is that the vibrator adaptor is always applied to the same site on the skull at repeated measurements.

CONCLUSIONS

The mechanical vibrations of the human skull, obtained by using vibrators of two different types, have been evaluated by means of a miniature accelerometer, rigidly attached to the skull, close to the cochlea. The vibrators were placed on different parts of the skull and fed with filtered clicks or short tone bursts. From the results obtained, the following conclusions can be drawn.

1. Filtered clicks should be avoided as stimuli in ECoG with bone-conduction stimulation. Tone bursts were found to be transferred to the skull in a superior fashion.

2. At 500 Hz the tone bursts were found to be converted to vibrations in a non-linear way, causing considerable distortion in the vibration recorded. This distortion is probably due to resonant phenomena in the skull itself, and may well be a possible source of error in conventional bone conduction audiometry as well.

3. Bone conduction stimulus levels increased by 10–25 dB when the vibr[illegible] were applied direct to the exposed [illegible] face of the mastoid process as [illegible]

with when applied across intact soft tissues This former way of application could be of advantage when using ECoG with bone conducted stimuli at ear surgery.

ACKNOWLEDGEMENT

We wish to express our gratitude to Prof Otto Grontoft, Department of Pathology, Regional and University Hospital, Linkoping, and his staff for giving us the opportunity to work in their department

ZUSAMMENFASSUNG

Die mechanischen Vibrationen im Knochen, der die Cochlea umgibt, wurden mit Hilfe eines Miniaturaccelerometers untersucht Ein Radioear B70A Vibrator und ein Brüel & Kjaer Mini Shaker wurden angewandt, stimuliert mit ‚filtered clicks' und mit kurzen „tone bursts , Tone bursts' waren besser als „clicks' in bezug auf das Vibrationsspektrum Bei 500 Hz konnte eine bedeutende Distorsion im Accelerometersignal, auch bei Anwendung von „tone bursts", observiert werden Es ist anzunehmen, daß diese Distorsion durch Resonanzvibrationen im Cranium verursacht wurde Deshalb ist hier mit einer Fehlerquelle zu rechnen, nicht nur bei der Anwendung von Stimuli mit kurzer Duration bei knochengeleitetem ECoG, sondern auch bei konventioneller, knochengeleiteter Audiometrie Wenn die Stimulationen direkt auf den Processus mastoideus appliziert wurden, war das Vibrationsniveau rund um die Cochlea 10–25 dB höher als wenn Weichteile den Stimulationspunkt deckten Diese Verhaltnisse ergaben Vorteile, welche vor allen Dingen bei ECoG wahrend einer Ohrenoperation genutzt werden onnen

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ELECTROCOCHLEOGRAPHY USED AS A CLINICAL HEARING TEST IN DIFFICULT-TO-TEST CHILDREN

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(Received December 8 1976)

Abstract Thirty difficult to-test children have been tested with transtympanic ECoG When possible informal hearing tests and/or free field testing were performed Children in whom no action potential (AP) could be recorded were submitted to conventional radiography of the inner ear and vestibular tests The correlation between free field test thresholds and AP thresholds was good especially in subjects with relatively good hearing Response amplitude increased and latency decreased with increasing frequency of the stimulus implying that different parts of the basilar membrane are stimulated according to the frequency of the stimulus Input–output curves of response amplitude and latency were plotted and three different types were distinguished ECoG can contribute to the evaluation of peripheral hearing in difficult to-test children and vestibular tests should always be performed on a child with suspected deafness or sensorineural hearing loss Conventional radiography of the inner ear however seems to be of little value

Electrocochleography (ECoG) is employed today in several centres for the clinical evaluation of the peripheral hearing It is a particularly valuable test when evaluating hearing in patients who refuse or are unable to cooperate in routine behavioral audiometry because they are very young, mentally retarded or emotionally disturbed

The purpose of this investigation was to study whether ECoG could elicit valuable information in the evaluation of hearing in difficult to-test children The intention was also to perform conventional audiometric tests when at all possible, and then to correlate these results with the ECoG results

This work was supported in part by Stiftelsen Tysta Skolan Stockholm Sweden

MATERIALS AND METHODS

Thirty children whose ages ranged from 1 to 16 years (mean 5 7 years) were tested There were 15 boys and 15 girls All children were referred because of a suspected hearing loss The etiology of the suspected hearing loss can be seen in Table I Eighteen of the children (60%) were mentally retarded

After an otological examination, conventional audiometric testing, including informal hearing tests and free field testing, was performed During informal hearing tests the child's reaction to sound from, for example, cow bells, drums and small bells is studied In free-field testing, acoustically calibrated sounds from similar sources, pure tones and narrow band noise are used when evaluating the hearing of a child Neither of these tests allows evaluation of each ear separately

Table I *Etiology of suspected hearing loss*

Rubella	5
Perinatal asphyxia	6
Meningitis	2
Encephalitis	1
Chromosomal aberration	3
Unknown	13

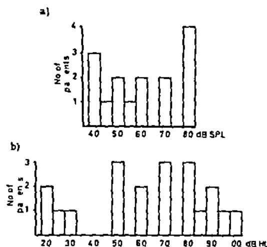


Fig 1 (*a*) Results of free field testing Reaction thresholds (dB SPL) Mean of 0 5 1 and 2 kHz (*b*) Results of AP threshold" determination Mean of 0 5 1 and 2 kHz

ECoG was done under general anesthesia using inhalation of halothane The children were pre medicated with atropine administered intramuscularly Induction was achieved by inhalation of nitrous oxide oxygen and halothane The children were intubated and anesthesia was maintained with nitrous oxide oxygen and halothane

The transtympanic technique was used The active electrode was of stainless steel 5 cm long and 0 2 mm in diameter It was placed ıstympanically on the promontory using a aural speculum under microscope con) The aim was to place this electrode half ay between the umbo and the annulus at approximately eight o clock (in a right ear) The electrode was secured with a rubber band on a doughnut placed circumaurally The ground and reference electrodes were either chlorided silver discs or subcutaneous platinum needles placed on the centre of the forehead and the lobe of the ear to be tested

The electrodes were connected to an amplifier (Grass P511 with HIZ511E high impedance probe) having a pass band of 30–3000 Hz and a gain of 50000 Summation of successive responses was done in an averaging computer (Intertechnique Didac 800) using 200 addresses in a time window of 10 msec

The stimulus consisted of short tone bursts presented in a series of 1000 at a repetition rate of approximately 20 per sec The rise and fall time of the bursts equalled one period of the tone frequency while the plateau duration was 6 periods or 4 msec whichever was the longer The tone frequency could be selected in octave steps from 0 5 to 8 kHz The stimulus level could be varied in 5 dB steps with a maximum of approximately 100 dB HL i e above behavioral threshold for the bursts in a normal hearing adult population

Response latency was read from a digital print out with an accuracy of 0 05 msec Response amplitude was read from an analogue XY recording with an approximate accuracy of 0 02 μV

In some cases where no action potential (AP) could be recorded and the patient was believed to be deaf or to have a profound sensorineural hearing loss the investigation was completed with conventional radiography of the inner ear and vestibular testing

Radiograms of the inner ear were studied with respect to malformations of the vestibule semicircular canals and cochlea A half axial frontal projection a Chausse III projection a lateral projection in 4 cases and in 1 case an axial projection were all used

The vestibular examination was performed as rotatory tests because of the age and status of these children using a MSA CF 10 centrifuge but did not allow evaluation of each side separately The following tests were performed (1) linear acceleration to the right and to the left (7 5°/sec² to 100°/sec) (2) deceleration to the right and to the left (7 5°/sec² from 100°/sec) and (3) oscillating rotation i e the angular velocity varies periodically in a sinusoidal pattern (amplitude 70°/sec acceleration 7 5°/sec²) During each rotation recordings were made using the technique of electronystagmography

RESULTS

Conventional audiometric tests

Informal hearing tests and/or free field tests were performed in 25 children In 5 cases no

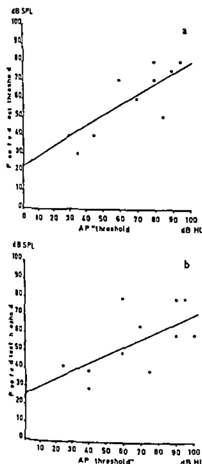


Fig 2 (*a*) Correlation between AP thresholds and free field thresholds at 2 kHz ($Y=0\ 6x+22\ 8$ $r=+0\ 77$) (*b*) Correlation between AP thresholds and free-field thresholds at 4 kHz ($Y=0\ 5x+26\ 2$ $r=+0\ 62$)

attempt was made to use these methods be cause of the child's severe mental retardation In 6 cases the result of the free field test was uncertain and no hearing threshold could be estimated In 4 cases only informal hearing tests could be performed Two children showed a moderate hearing loss and 2 a profound hearing loss The test results are shown in Fig 1*a*

Electrocochleography

In 10 patients no AP could be recorded at any frequency and any stimulus intensity They were classified as being totally deaf or having a profound hearing loss The other 20 children were placed in different groups according to the degree of hearing loss as determined by the mean AP "threshold" for 0 5, 1, and 2 kHz (Fig 1*b*) The 0 5 kHz tone burst very often produced such artifacts that it was im possible to evaluate whether an AP was pres ent or not An AP "threshold" at 0 5 kHz was identified in only 5 cases

The AP "threshold" was correlated with the free-field test threshold At 2 kHz the corre lation coefficient (r) was +0 77 ($p<0\ 01$) (Fig 2*a*) At 4 kHz the correlation coefficient was +0 62 ($p<0\ 05$) (Fig 2*b*)

The amplitude of the response at 90 dB HL varied between 0 4 μV and 13 8 μV The amplitudes at 90 dB HL for the different test fre quencies were correlated with the AP "threshold" (Fig 3*a–d*) The correlation coefficient ranged between −0 72 and −0 83 ($p<0\ 001$)

When amplitudes at 90 dB HL were plotted against frequency, no correlation was seen

The latency of the response at AP "thresh old" for two groups of AP "thresholds" was studied at different frequencies AP "thresholds" in the first group ranged between 15 and 40 dB HL and in the second group, 45–90 dB HL In the first group (Fig 4*a*) the correlation coefficient was −0 84 ($p<0\ 001$) and in the second group −0 47 ($p<0\ 01$) (Fig 4*b*)

Latencies at 90 dB HL plotted against frequency did not show any correlation For pa tients in whom responses were recorded at three stimulus intensities or more, the input–output curves of the AP amplitude and latency were plotted against stimulus intensity and studied Fig 5 (*a–c*) shows different types of input–output curves found among the children tested

Radiography

In 6 of the 10 patients where no AP could be recorded and the child was believed to be deaf, or to have a profound sensorineural hearing loss, conventional radiograms of the inner ear were taken Four children were impossible to examine because of their hyperactivity

The interpretation was difficult ...

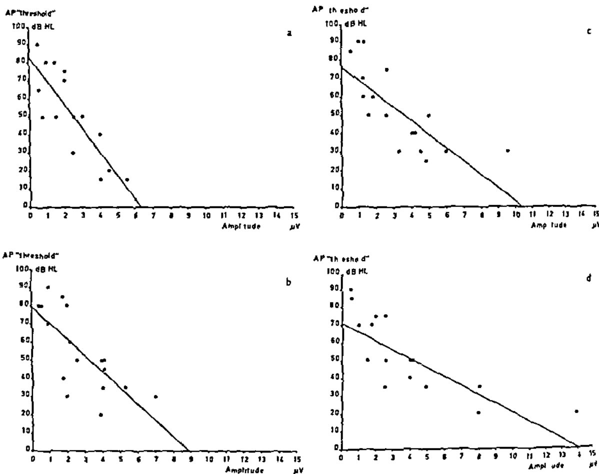


Fig 3 Correlation between amplitudes at 90 dB HL and AP "thresholds
(*a*) at 1 kHz ($Y=-13\ 3x+83\ 7$ $r=-0\ 83$)
(*b*) at 2 kHz ($Y=-8\ 9x+79\ 7$ $r=-0\ 72$)
(*c*) at 4 kHz ($Y=-7\ 2x+76\ 3$ $r=-0\ 76$)
(*d*) at 8 kHz ($Y=-4\ 9x+71\ 9$ $r=-0\ 78$)

adiologist but in all 6 cases no radiological ings suggestive of labyrinthine malformation were seen

Vestibular testing

Ten children in whom no AP could be recorded, were submitted to vestibular testing In 5 cases no nystagmus was recorded in response to the 3 rotatory tests which were performed In the other 5 children normal nystagmus was recorded The distribution of these patients with respect to etiology is shown in Table II

DISCUSSION

Commonly used audiometric tests for small children are informal hearing tests and free field testing In both types of test the child's reaction to acoustic stimulation is studied and from these reactions an attempt is made to evaluate the hearing threshold The advantage of free field testing compared with informal tests is that acoustically calibrated stimuli are used, enabling a more accurate estimation of the hearing threshold to be made A reaction threshold to acoustic stimulation at 30 dB sound pressure level (SPL) is considered normal

The disadvantage with both these tests is that they are very difficult to use on mentally retarded children or children with multiple handicaps In this study 5 children were impossible to test and in 6 cases the result was uncertain

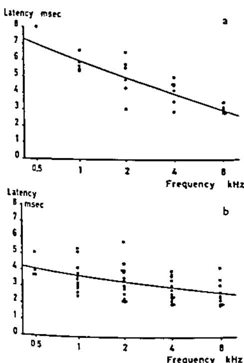


Fig 4 Correlation between latency at AP threshold and frequency
(*a*) For AP "thresholds" between 15 and 40 dB HL (Lat = −1.5 lnf+16.2, r = −0.84) (*b*) For AP "thresholds" between 45 and 90 dB HL (Lat = 0.5 lnf+7.4 r = −0.47)

In 10 patients tested with ECoG, no AP could be recorded at any frequency or any stimulus level. These children may be totally deaf, have a profound hearing loss or merely a severe hearing loss. This is explained by the fact that the behavioral threshold is usually better than the AP "threshold" (Hooper, 1973, Yoshie, 1973, Odenthal & Eggermont, 1974, Hooper et al., 1977.) Even though these studies were carried out on adults, one has to take into account that the subjective threshold may in extreme cases be up to 40 dB better than the AP "threshold". In practical clinical work it is probably wise to count with a subjective threshold 10 to 20 dB better than the AP threshold.

In this investigation the correlation between the free field test threshold and AP "threshold" for the better ear was studied at 2 and 4 kHz (Fig 2*a*, *b*). The correlation coefficients were good and show that the behavioral and

Fig 5 Input–output curves (at 2 kHz) (*a*) normal hearing (*b*) sloping amplitude–intensity curve (*c*) steep amplitude–intensity curve

AP thresholds correspond well at lower stimulus intensities but that the difference in favour of the behavioral threshold is greater at higher intensities. It should be pointed out, however, that the free-field threshold is expressed in dB SPL whilst the AP "threshol

Table II *Results of vestibular testing*

	Nystagmus	No nystagmus
Rubella	1	1
Perinatal asphyxia	1	1
Meningitis		1
Chromosomal aberration	1	
Unknown	2	2

is measured in dB hearing level (HL) which is roughly 10 dB less than dB SPL As thresholds to short tone bursts and to free field test sounds of longer duration are compared, temporal integration might affect the result (Pedersen, 1975) and give an imaginary better AP "threshold' at higher stimulus intensities However, this is not seen, the most likely explanation being that the reaction threshold in free field testing is more complex than an ordinary hearing threshold

According to the mean AP "threshold' 3 children had normal hearing (≤25 dB HL) (Fig 1*b*) Two of these were impossible to test with conventional methods and in 1 case the mean free field test threshold was 40 dB SPL

Low frequency stimuli are difficult to use in ECoG because (1) the point of maximum extation on the basilar membrane is remote the active electrode, (2) the firing among l elements is less synchronized (Davis, 6) and (3) stimulus artifacts are more often encountered The difficulty of testing lower frequencies creates a problem, since it is known that children with the types of hearing loss studied in this investigation usually have a sloping audiogram with the best hearing in the low frequency range This has to be considered when the results of an ECoG examination are evaluated

A good correlation was found between response amplitudes at 90 dB HL and AP "threshold' for the various test frequencies (Fig 3*a–d*) It is shown that the response amplitude at 90 dB HL increases with increasing frequency, the most likely explanation being that the AP is generated closer to the active electrode as the frequency increases and also the increased synchrony of firing among neural elements as the frequency is increased (Naunton & Zerlin, 1976) It is also seen that the larger the amplitude is at 90 dB HL the better is the AP "threshold' In practical testing this would mean that if a large amplitude were found at 90 dB HL, the hearing of the patient would be likely to be close to normal This observation is somewhat at odds with findings that patients with sensorineural hearing loss and recruitment often show normal or larger than normal amplitudes at high intensities and very steep amplitude intensity curves (Portmann & Aran, 1971*a*, Portmann & Aran 1971*b*, Aran, 1973, Portmann et al, 1973 Odenthal & Eggermont, 1974, Bergholtz et al 1977)

The fact that response amplitudes at 90 dB HL (when AP "thresholds" were near normal) increase with increasing frequency of the stimulus, shows that different parts of the basilar membrane are stimulated, depending on the frequency of the stimulus

Fig 4 (*a b*) shows that the latency of the response increases with decreasing frequency As the latency of the response is dependent on the distance between the oval window and the point of maximum stimulation on the basilar membrane, and the travelling wave velocity (Elberling, 1974, Naunton & Zerlin, 1976) this means that different parts of the basilar membrane are stimulated by different tone burst frequencies

The conclusion is that some frequency specificity exists and that it seems to be better at lower stimulus intensities (de Boer, 1975, Özdamar & Dallos, 1976) The relatively widely scattered values for amplitudes and latencies around the regression lines probably reflects different degrees of hearing loss

The fact that there seems to be some frequency specificity makes it possible to convert the AP "thresholds" to an "AP audiogram', taking into account the above discussed difference between behavioral and AP thresholds

Input–output curves of response amplitude

and latency are helpful in the differential diagnosis (Yoshie & Ohashi, 1969, Portmann & Aran, 1971*a*, Portmann & Aran, 1971*b*, Yoshie, 1973, Portmann et al, 1973, Odenthal & Eggermont, 1974, Bergholtz et al, 1977) In this investigation three types of input–output curves were found They represent normal hearing (Fig 5*a*), sensorineural hearing loss without recruitment (Fig 5*b*) and sensorineural hearing loss with recruitment (Fig 5*c*) according to the classification by Bergholtz et al, (1977) As stated earlier, none of the patients with a hearing loss of more than 30 dB HL showed larger than normal amplitudes at 90 dB HL The steepness of the amplitude intensity curve may reflect only the degree of hearing loss when amplitudes are expressed as a percentage of the maximum amplitude (Bergholtz et al, 1977)

Conventional radiographs of the inner ear seems to be of little value, since they are difficult to interpret and only major malformations can be seen Tomography would probably give more information (Jensen, 1969) but this examination has to be done under general anesthesia and the radiation dose is not insignificant in these young patients

Five of 10 children in whom no AP could be recorded did not show any vestibular reaction to rotatory tests It is known that children with deafness or profound sensorineural hearing loss may show abnormal vestibular reactions (Jensen, 1969) Vestibular tests should be included in the test battery when children with suspected sensorineural hearing loss are tested Preferably, caloric tests that permit each ear to be tested separately should be used if the child is old enough

In infants and small children, rotatory tests can be useful Abnormal or absent vestibular reactions in a child with suspected hearing loss increase the probability that the child really has a sensorineural hearing loss

CONCLUSIONS

Thirty children with suspected hearing loss have been tested with ECoG In some cases, informal hearing tests, free field tests, conventional radiography of the inner ear, and vestibular tests were performed The following are the main conclusions

(1) Informal hearing tests and free-field tests are very difficult to use on mentally retarded children and each ear cannot be tested separately

(2) ECoG is performed without cooperation of the child, under general anesthesia and each ear can be tested separately

(3) The behavioral threshold is often better than the AP "threshold" The correlation between free field test thresholds and AP "thresholds" was good The difference between the free-field test threshold and the AP "threshold" increases with increasing hearing loss

(4) Low frequency stimuli are difficult to use in ECoG This is a drawback, considering that most children with sensorineural hearing loss have a sloping audiogram with their best hearing in the low frequency range

(5) The response amplitude increases with increasing frequency, probably because the AP is generated closer to the electrode as the frequency increases and also because of increased synchrony of firing among neural elements This means that different parts of the basilar membrane are stimulated, depending on the frequency of the stimulus and that some frequency specificity exists

(6) The latency of the response (especially near normal AP "threshold") increases with decreasing frequency This also supports the theory that different parts of the basilar membrane are stimulated, depending on the frequency of the stimulus and that some frequency specificity exists

(7) If (3)–(6) are taken into account it is possible to convert the AP "thresholds" into an "AP audiogram"

(8) It is shown that the larger the amplitude is at 90 dB HL, the better is the AP "threshold"

(9) Input–output curves of response ... tude and latency can be used in the

tial diagnosis, although the correlation between steep amplitude–intensity curves and recruitment is not fully understood

(10) Conventional radiography of the inner ear seems to be of little value Tomography would probably contribute more but the radiation dose cannot be neglected

(11) Vestibular tests should always be performed on a child with suspected deafness or sensorineural hearing loss Abnormal or absent vestibular reactions probably support the theory that the child really has a hearing loss

ECoG can contribute to the evaluation of peripheral hearing in difficult to test children, i e very young and/or mentally retarded children However, its limitations have to be fully understood and in order to be able to make as correct a diagnosis as possible the examiner has to correlate the ECoG results with results from conventional tests This means that ECoG is a complementary hearing test and that, if at all possible, the conventional methods must be carried out There will always be a need for the skilful pediatric audiologist when it comes to evaluation of the hearing of a difficult to test child

ZUSAMMENFASSUNG

ig Kinder die schwer zu testen waren wurden mit panischer ECoG untersucht Wenn es moglich war auch informelle Gehörtests und Gehormessungen eien Feld durchgefuhrt In den Fallen wo kein Ak nspotential (AP) registriert werden konnte wurden Ubersichtsaufnahmen des Innenohrs wie auch Vestibularisuntersuchungen durchgefuhrt Die Korrelation zwischen Gehorschwelle im freien Feld und AP Schwellen war gut besonders bei niedrigem Re zpegel Die Amplitude nahm zu und die Latenzzeit nahm ab bei zunehmender Reizfrequenz Dies bedeutet daß verschiedene Teile der Basilarmembrane stimuliert werden was darauf beruht welche Reizfrequenz angewandt wird Die Input-Output Kurven wurden aufgenommen und hierbei konnten drei verschiedene Typen festgestellt werden Übersichtsaufnahmen des Innenohrs waren von untergeordneter Bedeutung Eine Vestibularisuntersuchung sollte immer durchgefuhrt werden bei einem Kind wo Taubheit oder sensorineuraler Horschaden vermutet werden ECoG gibt einen wertvollen Beitrag bei der Auswertung der peripheren Gehörfunktionen bei s hwer zu testenden Kindern

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PEROPERATIVE TEMPORARY THRESHOLD SHIFT IN EAR SURGERY

An Electrocochleographic Study

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Abstract TTS resulting from drilling in the temporal bone was detected by means of pre and post-exposure ECoG recordings taken during ear surgery for chronic otitis media The TTS obtained varied between 5 and 40 dB at 4 and 8 kHz The correlation between duration of noise exposure and magnitude of TTS was statistically significant The results indicate that drill induced noise in ear surgery may result in postoperative high tone sensorineural hearing loss and support the view that manipulation of the ossicular chain during ear surgery causes mainly a threshold shift at the lower frequencies

From two earlier studies by the authors (Kylen & Arlinger, 1976, Kylen et al , 1977) it is known that the surgical drill in ear surgery generates bone conducted noise levels of about 100 dB (equivalent airborne octave level) around the cochlea This type of noise has been found to have its main energy in the 2 and 4 kHz octave bands

Today, it may be regarded as an established fact that a noise level of this magnitude and frequency distribution is potentially harmful to the cochlea, the degree of the resulting noise trauma depending on the duration of noise exposure (Miller 1974) Drilling during ear surgery is often maintained for about one hour According to the same author (Miller 1974) a 2 4–4 8 kHz noise band at a level of 100 dB for one hour may produce a temporary threshold shift (TTS) at 4 kHz of about 40 dB, which is the TTS level at which a permanent threshold shift (PTS) may appear

The aim of the present study was to establish to what degree drill generated noise results in a TTS in patients submitted to ear surgery This required pre- and post exposure measurements of hearing thresholds under identical middle-ear conditions The only method available for estimation of the peripheral hearing in a patient under general anaesthesia is electrocochleography (ECoG) ECoG is an electrophysiological method in which, in response to acoustic stimuli, the compound action potential (AP) of the auditory nerve is recorded To facilitate these measurements we have developed a method of bone-conducted stimulation in ECoG (Arlinger & Kylen, 1977), which meets the special requirements of ear surgery, particularly sterility

MATERIAL AND METHODS

Fourteen patients with normal cochlear function (hearing thresholds by bone conduction at or below 25 dB HL ISO) were submitted ECoG during ear surgery Nine of th tients were exposed during surgery in the temporal bone for

Table I. *Cases 1–9, submitted between recordings to drilling in the mastoid for varying periods of time, in cases 10–14 no drilling took place*

Manipulations of the ossicular chain were performed between recordings in cases 3, 5, 6, 9, 12, 13, 14
D=Disarticulation of the incudostapedial joint H=Hammer dissection C=Columellization between the stapes and the drum +<5 min, ++<10 min, +++<15 min ×=No readings above background levels or disturbing stimulus artefacts *CAT*=Combined approach tympanoplasty *MP*=myringoplasty *TCT*=transcanal tympanoplasty *TTS*=temporary threshold shift *COM*=chronic otitis media

Case no	Diagnosis	Type of surgery	Duration of drilling (min)	Manipulation	*TTS (dB)* 2 kHz	4 kHz	8 kHz
1	COM	CAT	90	–	30	40	40
2	COM	CAT	75	–	20	20	30
3	COM	CAT	60	D+	10	10	10
4	COM	CAT	55	–	10	20	15
5	COM	CAT	50	D+	10	5	20
6	COM	CAT	40	D+	20	10	20
7	COM	CAT	40	–	×	20	30
8	COM	CAT	40	–	×	×	20
9	COM	MP	5	H++	20	10	10
10	COM Otosclerosis	MP	–	–	0	0	0
11	COM	MP	–	–	0	×	×
12	COM	MP	–	H+	0	0	0
13	COM	MP	–	H+++	20	0	0
14	COM	TCT	–	C+++	25	5	–5

minutes The remaining 5 patients were controls, in whom no drilling took place For further details, see Table I

The drilling equipment used was a Cyclone 160 with a rotation speed of approximately 16 000 rpm All the patients in this study were der general anaesthesia with thiopentone, O_2, intermittent pethidine and diazepam, scle relaxants with controlled ventila-

The methods used in this investigation differ to some extent from those usually applied in ECoG, and will therefore be described in detail

Stimulation

Short tone bursts, produced by a special stimulus generator, were used as stimuli As reported in a previous paper (Arlinger & Kylén, 1977), this type of stimulus when applied directly to the temporal bone by means of a bone conduction vibrator, has been found to be superior to filtered clicks The rise and fall-times of the bursts equalled one period of the tone frequency, while the plateau duration was 4 msec The tone frequencies used in the present study were 2, 4 and 8 kHz

The bone conduction vibrator employed was a Bruel & Kjaer type 4810 Mini Shaker A plexiglass rod (diameter 6 mm, length 60 mm) with a hemispherical tip was used as an adaptor and was screwed on to the vibrating

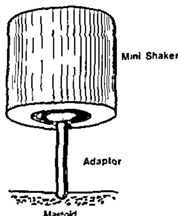


Fig 1 The adaptor of the Mini Shaker fitted into an excavation drilled into the cortical bone of the mastoid

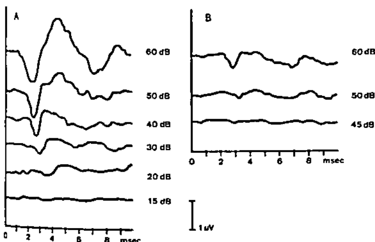


Fig 2 Calculation of TTS (8 kHz) for case 2 drilled for 75 min (A) AP threshold before noise exposure=20 dB (B) AP threshold after noise exposure= 50 dB TTS=30 dB Note increase in latency and decrease in amplitude in (B)

part of the Mini Shaker The Mini Shaker with the adaptor was firmly connected to the mobile arm of an operating microscope, permitting sterile dressing and a free choice of application angles and forces (by means of the focusing knob of the microscope) When using the vibrator, the sterile adaptor was placed in a small excavation drilled into the cortical bone of the mastoid (Fig 1) This procedure ensured that the vibrator was always placed on the same location on the skull The static application force used was approximately 5 N

The tone burst level could be attenuated in 5 dB steps Maximum attainable stimulus levels were 60–70 dB above normal behavioral thresholds for the tone bursts

Response recording

The electrical responses were picked up by means of a sterile, 0 2 mm diameter stainless steel needle electrode, placed on the exposed promontory, and a subcutaneous needle, placed in the contralateral temporal region Another subcutaneous needle in the contralateral cheek served as the ground electrode The signal was amplified in a Grass P511 amplifier with a HIP 511 E high impedance probe, using a gain of 50 000 and a pass band (−3 dB) of 30–3 000 Hz Summation of the responses to 1 000 stimuli with a repetition rate of approximately 20 Hz was performed by means of an Intertechnique Didac 800 digital averager, using 200 addresses and a time window of 10 msec The analog output of the averager was connected to an XY recorder, and the responses were recorded The response amplitude was determined from these recordings The digital ouput of the averager was connected to a digital printer, the printout of which permitted latency determination with an accuracy corresponding to the address time of the averager, 0 05 msec

Stimulating and recording

Before the surgical drilling was started and any ossicular manipulation was done, pre-exposure measurements were performed With all the equipment in place, the cochlea was stimulated with bone-conducted tone bursts at the three selected frequencies, and the hearing threshold for each frequency estimated In order to test the reproducibility of the method, the stimulation unit was removed and then replaced into the excavation of the mastoid A new recording was made and compared with an earlier one, obtained at the same frequency and intensity settings, with special regard to differences in the latency and the amplitude of the response This procedure was performed in 11 cases

When surgery had advanced to the po[int] when drilling was discontinued, the

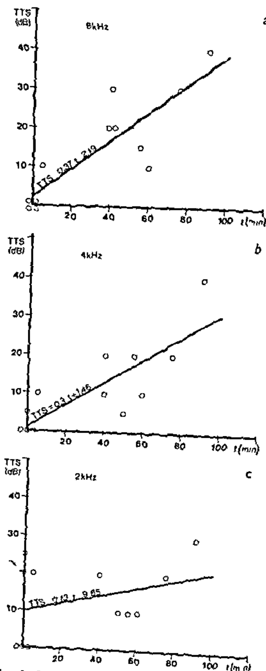


Fig 3 Correlation between the duration of noise exposure (=duration of drilling) and the TTS obtained (*a*) 8 kHz (*b*) 4 kHz (*c*) 2 kHz

imate duration of the drilling was recorded and a second determination of the thresholds was done, whereby the magnitude of the threshold shifts was obtained This second recording started approximately 5 min after the end of the noise exposure and lasted for 15–45 min In the control group, where no drilling took place, the patients were submitted to same type of procedure, the interval betw recordings being 45–60 min

Follow up

Postoperatively, all the cases were follow with audiograms, the first one taken when packing was removed one week after surger

RESULTS

The results of the present study are based comparisons between pre and post exposu electrocochleograms recorded during ear surgery In all, 14 patients were examined

The electrocochleograms have been analysed for shift of threshold, latency and amplitude

Threshold shift

In both pre- and post exposure ECoG recordings, the ECoG threshold was defined as the lowest stimulus level at each tone burst frequency that gave rise to an identifiable response Each of the three authors judged the thresholds independently Where disagreement in the judgements occurred, the threshold value giving the smallest TTS was used The TTS was defined as the difference in threshold levels between pre and post-exposure recordings Fig 2 illustrates this procedure In Table I it can be seen that all the patients subjected to drilling during surgery showed TTS at the three frequencies tested In 3 of the patients from the control group (cases 10, 11 and 12) no TTS could be found but 2 of them (cases 13 and 14) showed a TTS chiefly at the 2 kHz tone frequency Between the recordings in these two cases the ossicular chain had been manipulated for 10–15 min Fig 3 (*a–c*) shows the correlation between the duration of drilling and the TTS obtained at the three tone burst frequencies At 8 and 4 kHz the correlation coefficients were 0 87 and 0 82 respectively ($p<0.1\%$ and $p<1\%$) For the 2 kHz tone frequency the correlation coefficient was 0 41 (not statistically significant)

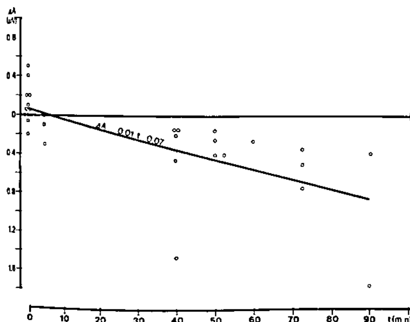


Fig 4 Correlation between the duration of noise exposure and the decrease in amplitude in the response 5–10 dB above AP thresholds (all frequencies)

Amplitude shift

The shift in amplitude has been defined as the difference in μV between the amplitudes of pre and post exposure recordings 5–10 dB above threshold Fig 4 shows the correlation between the duration of drilling and the decrease in amplitude (all frequencies) The correlation coefficient was 0 68 ($p<0$ 1%)

Latency shift

The shift in latency has been defined as the difference in msec between the latencies of the pre and post exposure recordings 5–10 dB

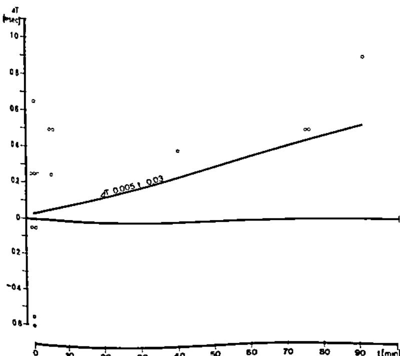


Fig 5 Correlation between the duration of noise exposure and the latency in the dB above AP thres (all frequencies)

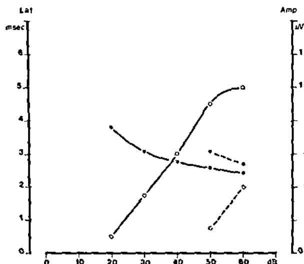


Fig 6 Case 2 Input–output function of amplitudes (O) and latencies (×) Decrease in amplitude and increase in latency are clearly shown Pre-exposure recordings (—) Post-exposure recordings ( )

over the threshold Fig 5 shows the correlation between the duration of drilling and the increase in latency (all frequencies) The correlation coefficient was 0 50 ($p<1\%$)

The decrease in amplitude and the increase in latency are also illustrated by the input–output functions of a typical case (Fig 6)

Test–retest reliability

T was no significant difference in the la y or the amplitude of the response after ing the stimulation unit and replacing it the same position (mean difference in latency 0 0273 msec S D =±0 152 msec and mean difference in amplitude 0 0318 µV, S D =±0 189 µV $n=11$ Student s t test)

Follow up

Case 1 (TTS during surgery 2 kHz 30 dB 4 kHz 40 dB, 8 kHz 40 dB) postoperatively was found to have a TTS detectable also in the pure tone audiogram (Fig 7) The hearing threshold returned to normal within one month All the other patients had postoperative bone conduction audiograms which showed no significant difference from their preoperative audiograms

DISCUSSION

The patients in this study all had chronic otitis media and satisfactory cochlear function The maximal attainable stimulation level was 60–70 dB above the behavioral threshold and a little less above the AP threshold A hearing threshold within 0–25 dB was therefore considered desirable in order to enable the recording of a TTS of 30–40 dB

The drilling equipment used was a Cyclone 160 In a previous study (Kylen & Arlinger 1977) this equipment was found to give noise levels in the order of 100 dB with a 6 mm cutting burr, and 3–11 dB less with 2–4 mm burr heads Thus, for most of the time, the cochlea was exposed to hazardous noise levels when the drill was used in the mastoid process One case in this study was drilled for 5 min and the rest for between 40 and 90 min The drilling in ear surgery is, for various reasons, interrupted from time to time We tried to make these interruptions as short as possible (<2 min) When forced by circumstances to break off the drilling for a longer period of time we preferred to make the second ECoG recording then and after that to continue the operation The duration of drilling given in Table I, include these minor interruptions, which can be expected to have reduced the TTS but, owing to the uneven distribution of the pauses it is impossible to estimate their effect on the noise trauma

All patients were under general anaesthesia

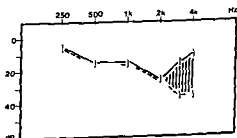


Fig 7 Postoperative TTS recorded in the bone-conducted audiogram of case 1 one week after surgery The hearing threshold returned to normal within one month Pre-operative audiogram (—) Post-operative audiogram ( )

With two exceptions the patients in the control group did not show a TTS and therefore the anaesthesia is considered not to have had any influence on the responses The 2 patients with a TTS in the 2 kHz tone frequency certainly had their TTS for other reasons Had anaesthesia caused this cochlear impairment, one would have expected the involvement of not only the 2 kHz frequency, but the other frequencies as well General anaesthesia is widely used in ECoG, but no drug influence on the compound action potential has been reported General anaesthesia may have other effects According to Rubinstein & Pluznik (1976), the incidence of cochlear damage from acoustic overstimulation in guinea pigs, is four times greater in an awake group than in one under general anaesthesia with Nembutal One gets the general impression that high tone, sensorineural hearing loss after ear surgery used to be more common when local anaesthesia was widely used in surgery for chronic otitis media Rubinstein's and Pluznik's report gives support to this assumption

The comparison of two separate ECoG recordings in the same patient necessitates a very reliable method There was no statistically significant difference between the amplitudes or the latencies of the tests and retests Studying the test–retest reliability in clinical electrocochleography Bergholtz et al (1976) found that the standard deviation of the differences between amplitudes of test and retest was 1 0 μV, and between latencies, 0 24 msec In the present study the standard deviation of the differences between amplitudes was 0 19 μV and between latencies, 0 15 msec We have therefore considered the test–retest reliability of our method to be satisfactory

For several reasons we have only recorded the compound action potentials in response to three tone burst frequencies in this investigation (2, 4 and 8 kHz)

(1) The higher frequencies are the more interesting ones when looking for a TTS resulting from broad band noise exposure

(2) ' The amplitude of response and the frequency selectivity of the stimuli are both strongly dependent on frequency Responses are clear and frequency selectivity is excellent at 4000 Hz and above, good at 2000 Hz adequate at 1000 Hz, poor at 500 Hz and useless at 250 Hz" (quoted from Davis, 1976) The explanation for this, given by Davis, is that the "response areas" of the cochlea are very frequency selective on the high frequency side, but not on the low-frequency side Moreover, Arlinger & Kylen (1977) found that the qualitative agreement between the tone burst output of the vibrator in bone conducted stimulation and the signal reaching the cochlea was excellent at high frequencies, but owing to distortion not so good at low frequencies

(3) The recordings were all done during surgery and the time spent obtaining them had to be kept as short as possible

A noise exposure of sufficient intensity and duration leads to a threshold shift The noise levels generated by the drill have their energy concentration in the 2–4 kHz bands (Kylen et al , 1976, 1977) These octave bands represent the frequency region in which the ear is most susceptible to noise (Miller, 1974) According to the same author, the TTS correlates with the duration of noise exposure, 100 dB for 5 min giving an average TTS of 15 dB, 40 min giving approximately 35 dB, and 60 min giving about 40 dB in the 4 kHz band, with errors from individual susceptibility excepted In this study we have found a statistically significant correlation between the duration of noise exposure and the TTS recorded in the 4 and 8 kHz bands (Fig 3*a* *b*) A noise exposure of 90 min gave a TTS of approximately 40 dB, one of 40 min gave 10–30 dB, and one of 5 min, 10 dB The variations in TTS obtained after the same periods of noise exposure should be due to variations in individual susceptibility to noise trauma The discrepancy between the TTS obtained and the TTS anticipated may result from the general anaesthesia and ı termittent character of the drill noise, tervals giving the cochlea time for covery The correlation between

tained and the duration of noise exposure was not statistically significant in the 2 kHz band (Fig 3c) The reason is that a TTS of 20–25 dB was recorded in 2 patients not submitted to drilling during surgery (cases 13 and 14, Table I) and that the patient with a noise exposure of only 5 min showed a TTS of 20 dB at 2 kHz and 10 dB at the higher frequencies (case 9, Table I) These patients had all been subjected to manipulations of the ossicular chain for 5–15 min between recordings

In the three other cases in the control group (cases 10, 11 and 12) the chain had either not been manipulated or had been manipulated for less than 5 min In these cases no TTS could be recorded Manipulation of the chain results in large, but presumably not very fast, deflections of the foot plate, causing pressure variations in the perilymph of the scala vestibuli These pressure variations seem to be more traumatic to the organ of Corti towards the apex of the cochlea, where the lower frequencies have their representation on the basilar membrane We find it reasonable to assume that manipulation of the ossicular chain during surgery was responsible for the TTS recorded in these control cases Studies concerning the effects of ossicular chain manipulation on the cochlea are in progress

Electrocochleographic supra-threshold cteristics of noise induced TTS are, ac- rding to Sohmer & Pratt (1975), an increase the latency and a decrease in the amplitude of the N_1-component of the AP (the first wave of the response)

In the present study, the correlation between the duration of the noise exposure and the decrease in amplitude and the increase of latency at stimulation levels 5–10 dB over the AP-threshold were statistically significant (Figs 4 and 5) We found a wide variation in the latency of response in the control cases (Fig 5) Close to the threshold the response is broad and the amplitude is small, rendering an accurate estimation of the latency more difficult (Bergholtz et al , 1976) In spite of this the correlation between the increase in latency and the duration of noise exposure was statistically significant

Case I (Table I) postoperatively was found to have a TTS detectable also in the pure tone audiogram (Fig 7) This was also the case in which the largest TTS values were recorded (40 dB at 4 and 8 kHz) and for whom the drill noise exposure was longest, 90 min

In their study of acoustic trauma of the cochlea from ear surgery, Schuknecht & Tonndorf (1960) stated that the acoustic intensities generated by the drill "apparently were at safe levels" Soudijn (1976), studying surface preparations of cochleas from drill-noise exposed guinea pigs, found distinctive lesions in the organ of Corti Soudijn's findings and the results of this study clearly indicate the possibility of a noise-induced PTS after drilling in ear surgery

As pointed out in a previous report (Kylen et al , 1977) the only way of minimizing the risk of cochlear trauma is to increase the cutting speed of the drill thus reducing the time of hazardous noise exposure to the inner ear

ZUSAMMENFASSUNG

P ocessus mastoi nach tion d 40 zeit Re

und Große von TTS war statistisch signifikant ... Re sultate ergaben auch den Grund zur Annahme daß Manipulationen der Gehorknochenkette während einer Ohren operation hauptsächlich TTS bei den niedrigen Frequenzen verursachen

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VOLUME DISPLACEMENT OF THE TYMPANIC MEMBRANE IN THE SITTING POSITION AS A FUNCTION OF MIDDLE EAR MUSCLE ACTIVITY

A Quantitative Microflow Method

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Abstract An open microflow meter system has been worked out for quantitative recording of the volume displacement of the tympanic membrane and its movement direction at stapedius reflex An acoustically elicited M stapedius contraction can be recognized by the characteristic response and latency time The stapedius reflex contraction causes an outward or inward movement of the tympanic membrane The magnitude of the volume displacement of the tympanic membrane is influenced by the middle ear pressure and in some ears the movement direction of the tympanic membrane changes

When a normal ear is exposed to a high sound pressure level, the middle ear muscles contract These contractions affect the ear in different ways attenuation of the transmission of [illegible] to the oval window, change in the [illegible] impedance in front of the tympanic [illegible] displacement of the ossicular chain and the tympanic membrane There is agreement in the literature that the contraction of M stapedius (N VII) is elicited acoustically, but whether the contraction of M tensor tympani (N V) is acoustically elicited is a moot point The middle ear muscles reflex responses have been recorded objectively in the following ways (1) recording of the change in the acoustic impedance in a closed ear canal and (2) recording of the change in the air pressure in a closed ear canal caused by tympanic membrane movements

A recordable outward movement of the tympanic membrane following acoustic stimulation has been regarded as an expression of a contraction of the stapedius muscle, while an inward movement of the tympanic membrane is said to be caused by a contraction of the muscle tensor tympani (Mach & Kessel, 1872 Mendelsson, 1957)

Terkildsen (1957) used a capillary manometer to show the direction of the tympanic membrane movements The investigation showed both outward and inward movements of the tympanic membrane, interpreted as contractions of both middle ear muscles during the acoustic stimulation The contraction of the M stapedius moved the tympanic membrane outwards and of the M tensor tympani inwards This pattern was confirmed by Holst et al (1963) recording the variations in the air pressure in an open and a closed ear canal In studies by Lidén et al (197[illegible]) impedance and pressure changes were recorded in the closed ear canal Yonovitz & Harris (1976) also made a study measuring the ear drum displacement with tympanometry and impedance

Lidén found that when an acoustic stimulus was produced, the reflex response was pri

These results were reported at Läkarsällskapets Riksstämma Stockholm 1975

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mainly caused by contraction of the M stapedius In 13% however the contraction of the M tensor tympani was suggested to be elicited acoustically According to Lidén the M tensor tympani pulls the tympanic membrane inwards while the M stapedius may pull the tympanic membrane in both directions though mostly outwards

Mendelsson (1966) recorded a pressure change at stapedius reflex response from positive to negative which he ascribed to changes in biophysic force vectors associated with the ossicular articulation

In an investigation of Bell s palsy Jepsen (1955) showed that a pure tone stimulus produced in the contralateral ear 75–115 dB above the hearing threshold was not followed by an impedance change ascribable to a contraction of the muscle tensor tympani This was confirmed by Terkildsen (1960) Klockhoff (1961) and Moller (1961)

In further investigations of the two middle ear muscles EMG recordings were performed by Salomon & Starr (1963) Djupesland (1965 1967) and Zakrisson et al (1974) These authors found that an acoustic stimulus elicited contraction of the M stapedius A contraction of M tensor tympani on the other hand was only observed when the acoustic stimulus was strong enough to cause a startle or defence reaction

Recording of the changes in acoustic impedance is the clinical method for investigation of the stapedius reflex response Metz (1946) introduced the mechanical acoustic impedance bridge while Morton & Jones (1956) Andersson et al (1956) Zwislocki (1957) Moller (1958) Terkildsen (1960) and others developed various electro-acoustic impedance bridges

Recordings with pressure or impedance in the ear can are both closed methods of investigation which are based both on the change in the stiffness or movement of the tympanic membrane and on the volume of the ear canal between the tympanic membran and the recording system These methods provide no possibility of measuring the volume displacement of the tympanic membrane Furthermore the impedance method does not allow recording of the direction of movement of the tympanic membrane With the open microflow method described in the present paper it has become possible to record quantitatively the influence of the middle ear muscle reflexes on the volume displacement of the tympanic membrane

The aim of the present authors has been to elaborate a method for quantitative recording of the volume displacement of the tympanic membrane and its direction of movement at M stapedius and M tensor tympani contractions As far as the stapedius reflex response is concerned the following factors have been established by use of this method

1) the acoustic reflex threshold
2) the latency and response time
3) the influence of the middle ear pressure

METHODS

Elner et al (1971*a*) worked out a microflow method for study of the compl ance of the tympanic system based on volume recording of the movements of the tympanic membrane by changing the ambient pressure in a pressure chamber With this method which implies free communication between the ear canal and the atmosphere it is possible to record the volume displacement of the tympanic membrane and the direction of its movements at middle ear muscle contraction Fig 1 provides an outline of the equipment used for the record ng A pressure chamber was used in which it was possible to change the pressure in the range +100 to −100 cm H_2O The adjusted pressure of the chamber (P_{ch}) was recorded by a differential pressure transducer

A flowmeter was connected with a cuff to the inner bony part of the external ear canal *The free end of the flowmeter opened int* pressure chamber After amplifying grating the flow signal it was poss b

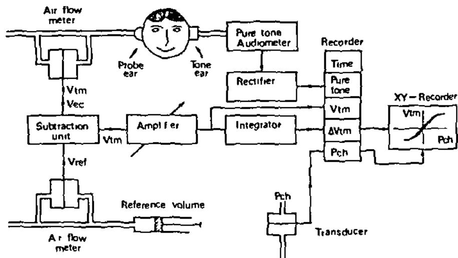


Fig 1 Schematic diagram of the equipment Symbols V_{tm} Air flow velocity through the resistor of the ear canal flowmeter caused by the volume deviation of the tympanic membrane V_{ec} Air flow velocity through the resistor of the ear canal flowmeter caused by compression or expansion of the gas in the external ear canal and in the flow meter system by change in the ambient pressure V_{ref} Flow velocity through the resistor of the reference flow meter caused by compression or expansion of gas in the reference system by change in the ambient pressure P_{ch}, Pressure in the pressure chamber i e the ambient pressure V_{tm} Volume deviation of the tympanic membrane in relation to its neutral position Δ before the symbol indicates a change of the variable

the volume displacement of the tympanic membrane (ΔV_{tm})

In order to record the volume displacement of the tympanic membrane correctly even at small variations in the ambient pressure and temperature, an identical flowmeter was used which was connected to an adjustable reference volume system In the case of volume balance between the two flow systems, the ambient flows V_{ec} and V_{ref} are identical (Fig 1), and after subtraction the isolated flow recording is caused only by the movements of the tympanic membrane, V_{tm} The sensitivity of the flow amplifier can be adjusted so that an integrated air-flow volume of 0 01 μl through the flowmeter causes a deflection of 1 mm on the inkjet recorder An X–Y recorder was used for recording the volume deviation of the tympanic membrane (V_{tm}) with the Eustachian tube closed, as a function of the change in ambient pressure (P_{ch}), i e the volume pressure relationship of the tympanic membrane For more detailed data, see Elner et al (1971*c*)

An audiometer (Tegner T-2) with earphones (TDH-39) was used as acoustic pure tone stimulator for the triggering of the middle ear muscle reflex response The rise time of the pure-tone was 0 3 dB/ms with stimuli intensity 75–105 dB and the duration was operated manually with the interrupter of the audiometer The signal of the stimuli intensity was recorded on an inkjet recorder after rectification The audiometer was calibrated according to ISO, 1964

MATERIAL

Only such subjects were selected as could completely and easily equilibrate the middle ear pressure to the atmospheric pressure All the subjects belonged to tubal function group Ib *ad modum* Elner et al (1971*b*) Group Ib can equilibrate relative over- and underpressure ±10 cm H_2O completely by three deglutitions

The material consisted of 10 persons (6 women and 4 men), 22–44 years of age, who

Table I *Hearing thresholds of air conduction in a sitting position with the tympanic membrane in its neutral position in dB rel ISO 1964*

+=hearing loss −=hearing gain

Case	Left				Right			
	500 Hz	1 kHz	2 kHz	4 kHz	500 Hz	1 kHz	2 kHz	4 kHz
I	+5	−5	−5	+5	0	0	−5	+10
II	0	0	+5	0	+5	0	+10	+5
III	No rec	No rec	No rec	No rec	+10	0	+5	+5
IV	−5	−5	−5	0	0	0	−5	+5
V	0	−5	−5	0	0	−5	−5	0
VI	0	0	0	0	+5	+10	0	0
VII	+10	+5	0	−10	0	0	+5	−10
VIII	+5	0	−5	+5	+10	−5	−5	+5
IX	0	0	−5	0	0	0	−5	+20
X	0	0	−5	+10	+5	+5	−5	+15

had no history of ear disease and who on ear microscopy were found to have normal ear drums Nineteen ears were examined, one ear was excluded because of reduced Eustachian tube function

PERFORMANCE AND RESULT

I *Hearing Threshold Determination*

(a) *At middle ear pressure the same as the ambient pressure*

The absolute hearing thresholds for bone as well as air conduction coincided and were examined with conventional audiometry (ISO 1964) The hearing thresholds for air are seen in Table I

(b) *At various middle ear pressures*

By establishing the absolute hearing thresholds at different over- and underpressures in the middle ear we were able to study the influence of the pressure on the sound transmission to the inner ear This is very important as variations in the middle ear pressure can affect the stapedius reflex response

The different middle ear pressures were produced via the Eustachian tube through pressure application in the rhinopharynx by deglutition, and the middle ear pressure was checked by conventional tympanometry (Madsen Z072) The hearing threshold was determined by Bekésy audiometry (fixed frequency technique at 1 kHz) at different known relative middle ear pressures 0, ±2, ±10, ±15 cm H_2O These examinations will show that there is a gradual hearing loss at increasing positive as well as negative middle ear pressure (Table II) for this frequency

II *Compliance of Tympanic Membrane*

We have studied the complicance by recording the degree of volume displacement of the tympanic membrane, V_{tm}, with the Eustachian tube closed at different chamber pressures, P_{ch} in the range +15 to −15 cm H_2O according to Elner et al (1971c) It is important to find out whether there is a correlation between the compliance and the volume displacement at the stapedius reflex response

The results are presented in Fig 2, where

Table II *Change in hearing threshold at 1 kHz in dB at different middle ear pressures as P_m in cm H_2O as compared with hearing threshold at P_{atm} in the middle ear investigated in 19 years*

P_m/cm H_2O	+15	+10	+2	−2	−10	15
Mean dB	6 7	5 8	2 2	2 1	5 2	6 8
S D dB	3 8	3 6	1 6	1 0	2 8	3 7
S E dB	1 2	0 9	0 4	0 3	0 7	0 9

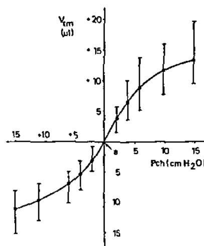


Fig 2 Compliance of the tympanic membrane Neutral position=origo (*a*) at P_{ch}=0 cm H_2O The outward movement of the tympanic membrane is recorded in the right upper quadrant and the inward movement in the bottom left quadrant (V_{tm} in μl) The dots represent the mean value for the whole material (19 ears) and the columns represent the range

the mean deviation of the tympanic membrane is reported at different chamber pressures The solid line represents the mean volume displacement of the tympanic membrane in relation to its neutral position

Comments There is no significant difference (p>0 05) in the volume pressure relation of the tympanic membrane in our material as ..d with Elner et al (1971*c*)

III *Influence of Stapedius Reflex Response on Tympanic Membrane in its Neutral Position*

(*a*) *Volume displacement of tympanic membrane*

The stapedius reflex response was recorded as a volume displacement of the tympanic membrane when a pure tone stimulus was produced in the contralateral ear with the subject in a sitting position and the middle ear pressure equal to the ambient pressure

There is a regular outward or inward movement of the tympanic membrane characteristic of each ear From the representative examples recorded it appears that the tympanic membrane moves synchronously with the pulse (Fig 3*a*)

At pure tone stimuli 1 kHz 105 dB, case shows an outward movement of the tympanic membrane, while case VI shows an inward movement The upper curve (V_{tm}) represents the recorded flow velocity caused by the outward (*b*) or inward (*c*) movement of the tympanic membrane, while the lower (ΔV_{tm}) represents the integrated flow velocity corresponding to the volume displacement of the tympanic membrane

The figure shows that the tympanic membrane deviates more from its neutral position at the onset of the stimulus (overshoot) In the following discussion these two phases of the volume displacement will be called the initial (*i*) and the permanent (*p*) (Fig 3)

The values presented in Table III are recorded at 1 kHz, 105 dB in the sitting position and represent the mean values from 30 recordings from each ear of the subjects There is slight variation, especially as regards the initial volume displacement Assessment of the permanent volume displacement (*p*) is somewhat uncertain, as pulse and breathing blur the recording

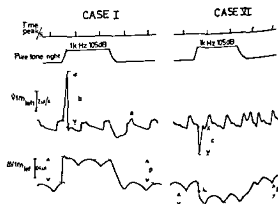


Fig 3 Recording in two cases V_{tm} represents the recorded flow velocity at an outward (*b*) or inward (*c*) movement of the tympanic membrane and movements of the tympanic membrane synchronous with the pulse (*a*) ΔV_{tm} represents the integrated flow velocity corresponding to the volume deviation of the tympanic membrane *i*=initial *p*=permanent phase of the volume displacement of the tympanic membrane at stapedius reflex contraction

Table III *Initial and permanent volume displacement of the tympanic membrane* ($\Delta V_{tm_{stap}}$) *in µl at pure tone stimulus 1 kHz 105 dB produced in the contralateral ear rel ISO 1964 with the tympanic membrane in a controlled neutral position and the subject in a sitting position*

+ outward movement of the tympanic membrane − = inward movement

	Left (probe ear)						Right (probe ear)					
	Initial $\Delta V_{tm_{stap}}$			Permanent $\Delta V_{tm_{stap}}$			Initial $\Delta V_{tm_{stap}}$			Permanent $\Delta V_{tm_{stap}}$		
Case	Mean	S E	S D	Mean	S E	S D	Mean	S E	S D	Mean	S E	S D
I	+0 515	0 007	0 039	+0 461	0 010	0 054	+0 376	0 007	0 038	+0 338	0 009	0 048
II	+0 351	0 008	0 043	+0 341	0 007	0 037	+0 454	0 007	0 038	+0 428	0 007	0 037
III	+0 381	0 009	0 051	+0 374	0 010	0 053	No rec	No rec	No rec	No rec	No rec	No rec
IV	+0 111	0 007	0 043	+0 106	0 008	0 046	+0 044	0 002	0 009	0	0	0
V	+0 058	0 005	0 025	+0 041	0 008	0 034	+0 212	0 005	0 025	+0 173	0 005	0 026
VI	−0 372	0 009	0 051	−0 284	0 008	0 045	−0 349	0 005	0 028	0 251	0 005	0 027
VII	+0 108	0 006	0 032	+0 099	0 005	0 027	−0 154	0 006	0 033	−0 115	0 006	0 033
VIII	+0 118	0 004	0 021	+0 102	0 004	0 022	+0 035	0 002	0 008	+0 027	0 004	0 021
IX	+0 536	0 009	0 050	+0 502	0 009	0 050	+0 288	0 004	0 030	+0 264	0 004	0 021
X	−0 065	0 002	0 011	−0 015	0 003	0 020	−0 104	0 004	0 020	−0 023	0 005	0 027

Comments There is a tendency towards correlation between the volume displacement of the tympanic membrane and the compliance, i e the more mobile the tympanic system, the greater the stapedius reflex response The measurements are based on the initial phase of the volume displacement of the tympanic membrane in the neutral position ($\Delta V_{tm_{stap}}$) at 1 kHz, 105 dB as a function of the mobility of the tympanic membrane (ΔV_{tm}) recorded in a pressure range +15 to −15 cm H_2O The tympanic membrane moves outwards for most of the ears (14 ears) with the correlation $\Delta V_{tm_{stap}} = -0\ 014 + 0\ 011\ \ \Delta V_{tm}$ and the regression coefficient 0 22 When the tympanic membrane moves inwards the correlation $\Delta V_{tm_{stap}} = -0\ 116 + 0\ 012\ \ \Delta V_{tm}$ and the regression coefficient 0 42

As far as the direction of the movement and the size of the stapedius reflex response are concerned, there are great individual differences, but the difference between the ears of the same subject is considerably smaller and only in one subject could opposite directions of movement be recorded, viz case VII

b) Acoustic threshold of stapedius response

The reflex response was caused by pure tone stimuli produced in the contralateral ear at a stimulus intensity of 80–105 dB at the frequencies 500 Hz, 1 kHz, 2 kHz and 4 kHz Table IV) The stimulus intensity was increased in stages of 5 dB until the acoustic threshold was reached (i e the moment when the reflex response deviated 0 1 µl/sec from the original recording of the flow)

Comments We have chosen to record the threshold related to the normal hearing threshold according to ISO 1964 For assessing the threshold of the stapedius reflex response of each ear, correction can be done according to the corresponding hearing threshold (Table I)

(c) Influence of stimulus intensity on stapedius reflex response

During the examination of the volume displacement of the tympanic membrane at different stimulus intensities above the acoustic threshold of the stapedius reflex response up to 105 dB, it was found that 75% of the ears showed no increase of the volume displacement above 95 dB, while the rest 25% showed an increasing volume displacement up to 105 dB (Fig 4)

(d) Latency time of stapedius reflex response

Definition The difference in time between the beginning of the sound stimulus (i e the

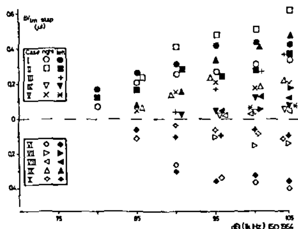


Fig 4 The volume displacement of the tympanic membrane at acoustic stimulus produced in the contralateral ear at intensities 75–105 dB according to ISO 1964 and at the frequency 1 kHz The material consisted of 19 ears and each symbol represents the mean value of two recordings for each ear at each level of intensity (+=outward movement of the tympanic membrane −=inward movement)

ment when the sound stimulus has reached the adjusted stimulus intensity of the audiometer) and a recordable reflex response (i e the moment when the reflex response has diverged 0 1 μl/sec from the original recording of the flow) We have estimated the latency time both at the threshold of the stapedius reflex response and at a stimulus 5 dB above the threshold The result appears from Table V

omments As we have used a stimulus intensity increase of 0 3 dB/ms we have not beer able to estimate the latency time for higher intensities than 5 dB above the hearing threshol as the latency time decreases with increasin stimulus intensity

In 3 subjects (5 ears case II left, III and IV bilaterally) two reflex responses have beer recorded (1–2 times per 100 stimulations) with varying latency time for the same acoustic stimulus (1 kHz, 105 dB), (cf the example of recording, Fig 5) In these ears the second delayed reflex response, which was very rare always shows a vigorous inward movement of the tympanic membrane, interrupting the permanent phase of the stapedius reflex response independently of the tympanic membrane is placed inward or outward related to its neutral position We interpret these later reflexes as caused by musculus tensor tympani contractions (*c, d*) The mean value for the latency of later reflex is 650 ms and the range 300–1 200 ms The volume displacement of M tensor tympani cannot always be measured as the volume amplitude is often too great to be recorded

(*e*) *Rise time of the M stapedius reflex response*

Definition The length of time from a recorded stapedius reflex contraction until 63% of the

able IV *Threshold of the stapedius reflex response recorded as the vol me displacement of the tympanic membrane* $\Delta V_{tm_{stap}}$, *in the sitting position by pure tone s* *r the contralateral ear at the neutral position of the tympanic membrane in*

Case	Left (tone ear)				Right (tone ear
	500 Hz	1 kHz	2 kHz	4 kHz	500 Hz
I	90	85	80	95	90
II	85	85	90	100	85
III	No rec	No rec	No rec	No rec	90
IV	95	95	95	100	90
V	90	85	80	80	100
VI	85	85	80	95	85
VII	100	95	>105	>105	105
VIII	100	95	95	100	100
IX	80	85	95	100	80
X	90	90	90	90	85
Mean	90 6	88 9	88 1	95 0	91
S D	6 8	4 9	7 0	7 0	8
S E	2 3	1 6	2 5	2 5	2

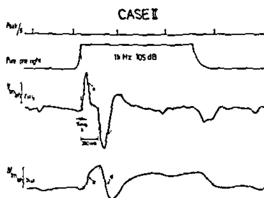


Fig 5 Example of recording the flow velocity (V_{tm}) (*a c*) and the volume displacement of the tympanic membrane (ΔV_{tm}) (*b d*) *a* and *b* means an outward movement of the tympanic membrane caused by contraction of M *stapedius* while *c* and *d* is an inward movement of the tympanic membrane probably caused by contraction of M tensor tympani

maximum volume displacement has been reached The results are shown in Table V

Comments We have only been able to estimate the rise time of six ears, where the volume displacement of the tympanic membrane is greater than 0 3 μl When the volume displacement is smaller the rise time cannot be estimated reliably because of the pulse synchronous movements of the tympanic membrane, which interfere with the recording

IV *Volume Displacement of Tympanic Membrane at Stapedius Reflex Response at Different Positions of Membrane*

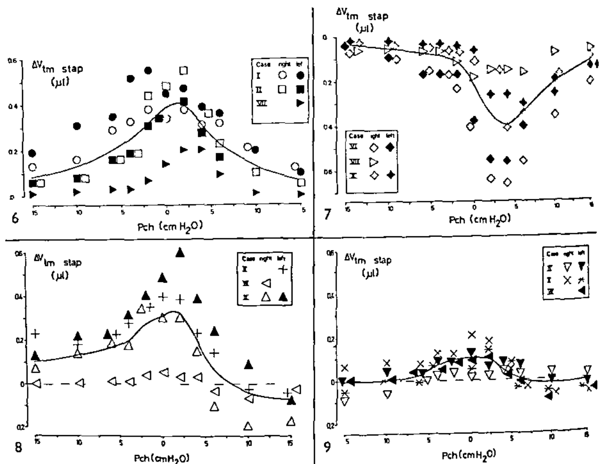
An acoustic stimulus 1 kHz, 105 dB was selected as providing the greatest reflex response when the tympanic membrane is in its neutral position With the aid of a pressure chamber the ambient pressure around the subject was changed in the range +15 to −15 cm H_2O In that way the tympanic membrane was placed in different constant positions outside or inside its neutral position when the Eustachian tube was closed The pressure difference across the tympanic membrane can be considered to be of the same magnitude as the pressure change in the chamber with a normal middle ear volume (Elner et al , 1971*c*) The stapedius reflex response was recorded at the pressures P_{atm}, ±2, ±4, ±6, ±10, ±15 cm H_2O

The results of the stapedius reflex response at different positions of the tympanic membrane can be divided into four different groups Group I always shows outward movements of the tympanic membrane and group II constantly inward movements at the different positions of the tympanic membrane Groups III and IV show outward movements but when the tympanic membrane is outside its neutral position the direction of movement changes to an inward movement The division into groups III and IV is based on differences due to changes in posture (Casselbrant et al , 1977)

In Fig 6 the results of the volume displacement at stapedius reflex contraction from 5 ears (3 subjects) are presented, all of which show an outward movement irrespective of the position of the tympanic membrane The greatest reflex response is recorded when the tympanic membrane is kept a little outside its neutral position (P_{ch}−2 cm H_2O) (cf mean value curve)

Table V *Latency and rise time of the stapedius reflex response at 1 kHz pure tone stimulus at various intensities above the individual threshold of the stapedius reflex*

	0 dB	+5 dB	+10 dB	+15 dB	+20
Latency time (19 ears)					
Mean ms	107	66			
Range ms	50–230	30–140			
Rise time (6 ears)					
Mean ms	150	101	82		
Range ms	80–330	50–180	50–110		



Figs 6 9 The volume displacement of the tympanic mem brane at stapedius reflex at different positions of the tympan c membrane Each symbol represents the mean value from three recordings in each position The solid gives the mean value The stimulus produced to lateral ear 1 kHz 105 dB *Fig 6* Only outward movements at stapedius reflex contraction irrespective f he on f he t mpan c membrane *Fig 7* Only

membrane

In Fig 7 the volume displacement in 5 ears (3 subjects) is presented All ears showed an inward movement of the tympanic membrane irrespective of the position of the tympanic membrane The greatest stapedius reflex re sponse is recorded when the tympanic mem brane is outside its neutral position (P_{ch} 4 cm H_2O) (cf the mean value curve)

In Figs 8 and 9 the volume displacement in 9 ears (6 subjects) is presented When the tym panic membrane is outside its neutral position there is a change in the movement direction from an outward to an inward movement In Fig 9 some of the ears also show a change in movement direction when the tympanic mem brane is inside its neutral position The great est stapedius reflex response is recorded in group III (Fig 8) when the tympanic mem brane is outside its neutral position (P_{ch} −2 cm H_2O) and in group IV when it is in its neutral position (Fig 9)

Comments When the chamber pressure is changed, there is a difference across the tympanic and the sion properties fected (Table duction, h only d Fig 4

DISCUSSION

With the open microflow method it has become possible for the first time to record quantitatively the volume displacement of the tympanic membrane at stapedius reflex contraction independently of the volume of the auditory canal between the cuff and the tympanic membrane This means that we can compare the stapedius reflex response recorded during different investigations and also see how the stapedius contraction affects the volume displacement of the tympanic membrane in different known membrane positions Thanks to the d c recording from the flowmeter we can record the direction of movement of the tympanic membrane and confirm the results of other authors, such as Mendelsson (1966) and Liden et al (1970), i e that the M stapedius can pull the tympanic membrane outwards as well as inwards

The transient response time (63%) of the flowmeter *system in our investigation is 10 ms* This seems enough to distinguish an acoustically elicited M stapedius contraction from a supposed M tensor tympanic contraction (Fig 5) on the basis of the different latency times of the reflexes, as has also been demonstrated by Djupesland (1965) and others using EMG recordings

Impedance at probe frequency and pressure recordings in the auditory canal are closed methods which do not admit of quantitative recording of the volume displacement at stapedius reflex contraction

When measuring the threshold of the stapedius reflex contraction with the impedance method by pure tone stimulation in the contralateral ear, Jerger et al (1972) found that the reflex thresholds for a normal ear are about mean 85±8 dB (S D) above the hearing threshold in the frequency range 250–2 000 Hz In the present investigation, we found the threshold at mean 89 8±8 2 dB (S D) above the hearing threshold at the frequency range 500–2 000 Hz This shows that the sensitivity of the microflow method is about the same as that of the impedance method in recording a stapedius contraction

From the present investigations it appears that the magnitude of the stapedius reflex response is dependent on the middle ear pressure The greatest reflex response is reached when there is a slight overpressure (about 2 cm H_2O) in the middle ear, i e the tympanic membrane is outside its neutral position The response is reduced by increasing under- and overpressure in the middle ear, and may even sometimes totally disappear (Figs 6–9) This shows the importance of checking the middle ear pressure in measurements of the clinical stapedius reflex threshold

In our investigation of the latency time of the stapedius reflex response we found a mean value of 107 ms at stapedius reflex threshold and the latency time decreased with increasing stimulus intensity This agrees with the findings of Metz (1951), Moller (1953) and Liden et al (1970) Since the stimulus intensity increase *was 0 3 dB/ms it was only possible to estimate* the latency time for the threshold and at 5 dB above the stapedius reflex threshold

An exact comparison between estimated latency times and rise times as given by other authors is difficult, as the onset of the stimulus intensity and the definitions of the latency time and the rise time are not always reported

In the present investigation the stapedius reflex response is characterized as an outward or inward movement of the tympanic membrane which remains as long as the acoustic stimulus is going on In five ears, at a strong acoustic stimulus (105 dB), we have only on a few occasions recorded a vigorous inward movement of the tympanic membrane at a latency time of about 300–1 200 ms We presume that this late reflex response is caused by a M tensor tympani contraction We have not checked if this reflex response is correlated to a palpebral reflex On comparison, Djupesland (1965) found that this reflex response was elicited at a higher stimulus intensity and that the latency time was 80–2 ms

During the transient phase following the stapedius reflex response at an acoustic stimulus a greater volume displacement of the tympanic membrane is obtained initially (overshoot) than at the continuous permanent outward or inward movement of the tympanic membrane (Fig 3) The reason for recording these two phases is that the inner ear will primarily be exposed to a vigorous acoustic stimulus before the M stapedius has reached its maximal contraction Owing to the moderating transmission qualities a lower sound pressure reached the inner ear and the muscle contraction decreases

Moller (1961) calls this "the amplitude regulation of the acoustic reflex" comparing the middle ear transmission to a servo system, whose transients are more pronounced at low frequencies owing to the greater moderating abilities of the M stapedius at these frequencies

As stated earlier, the M stapedius pulls the tympanic membrane outwards as well as inwards and the direction of strain is characteristic of each ear when the tympanic membrane is in its neutral position In the course of the investigation of the M stapedius at the different middle ear pressures we have found that the direction of strain of the tympanic [illegible] can be changed from an outward [illegible] an inward movement when the tympanic [illegible] ne is outside its neutral position In some ears, however, this also occurs even when it is inside its neutral position This change in the movement direction of the tympanic membrane at the various middle ear pressures is always constant in each ear

The principal aim of the present authors has been to measure the volume displacement of the tympanic membrane quantitatively at stapedius reflex contraction and to find out what middle ear variables influence the stapedius reflex response It will be investigated in a later paper whether variations in the inner ear pressure can be measured on the outside of the tympanic membrane at stapedius reflex contraction, if the middle ear variables can be controlled Work is in progress to explain the various movement patterns of the tympanic membrane at stapedius reflex contraction

ACKNOWLEDGEMENT

The authors are grateful to Ass Prof Henry Andersson Department of Audiology Karolinska Sjukhuset Stockholm for his valuable discussion and constructive suggestions

ZUSAMMENFASSUNG

Fur die quantitative Registrierung der durch Lageveranderungen des Trommelfells verursachten Volumenschwankungen im Gehorgang sowie die Richtung de[illegible]

[illegible]

tenzzeit erkannt werden Die durch Stapediusreflex he[illegible] vorgerufene Kontraktion erzeugt eine Bewegung d[illegible] Trommelfells in der Richtung nach außen oder auch nac[illegible] innen Die Große der volumenbedingten Lageveranderu[illegible] des Trommelfells ist vom Druck im Mittelohr abhängi[illegible] und an manchen Ohren andert sich die Bewegungsric[illegible]tung des Trommelfells

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BILITY OF LEVEL-DIAGNOSTIC EXAMINATIONS N ACUTE, PERIPHERAL FACIAL PALSY

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Abstract In acute non traumatic peripheral facial palsy where the cause is not known it does not seem to be justified to draw conclusions as to how far to the centre in the course of the nerve the lesion may be located based on an examination of tear secretion stapedial reflex and taste This is demonstrated in two cases

In 1829 Sir Charles Bell claimed that the muscles of the face are controlled not by a branch of the trigeminus, but by a separate nerve His observations on facial palsy were founded on injuries caused by a pistol wound in the region of the ear, by the horn of a bull entering under the angle of the jaw, and by removal of prae auricular tumor Thus the sites of these nerve lesions were reliably es-
shed Since then, peripheral facial palsy often been called Bell's palsy, but in the past this description has been restricted ostly to a group of non traumatic, acute peripheral facial palsies in which the cause and site of the lesion is not always known As the aetiology and the pathogenesis of the condition have been ascribed to many factors (Kettel, 1959, Taverner, 1965 Djupesland et al., 1975) it is not surprising that very differing kinds of treatment have been tried and abandoned (Wolferman, 1974) The most persisting form of treatment is the decompression operation Usually the facial nerve is not exposed centrally to the ganglion geniculi However, if such an operation is to be carried out, the site of the lesion must be assumed to be at a place accessible to surgery

Some authors (Taverner, 1965, Stien & Tonning, 1973) assume that in Bell s palsy examinations of tear secretion (Schirmer test ref Zilstorff Pedersen, 1965) it is the stapedia reflex (Jepsen, 1955) and taste (Krarup 1958 that give an indication of the seriousness an extent of the injury, rather than of the site since disturbances of all of the level diagnosin functions mentioned were more often found i clinical paralyses than in pareses This scepti cism regarding the traditional attitude to leve diagnosis has been strengthened by the follow ing observations

Case 1

Woman, 38 years old Three days after th onset of a sudden right sided headache, sh suffered an acute peripheral facial paralysis o the same side Tear secretion on the affecte side was 10 mm, on the other side 20 mm after 5 minutes The stapedial reflexes wer normal and symmetrical The results by elec trogustometry were so variable that no conclu sion could be drawn Neurological examina tion showed nothing pathological except th facial paralysis The spinal fluid was found t be normal

Case 2

Man, 50 years old, operated for a right side acoustic neuroma In connection with th operation the facial nerve was exposed to slight tension in its intracranial course Th

patient suffered a light, peripheral facial palsy, which still persisted on examination 2 years after the operation The tear secretion on the affected right side was still reduced, 4 mm, on the left side, 17 mm However, the stapedial reflexes and the sense of taste were still completely normal and symmetrical

DISCUSSION

When the sense of taste is disturbed by peripheral facial palsy, the injury to the facial nerve is believed to be located central to the point at which the tympanic chord branches off If the stapedial reflex is disturbed the lesion is assumed to be central to the stapedial nerve If the tear secretion is reduced the lesion is assumed to be central to the geniculate ganglion

When a facial palsy is attributed to loss of continuity of the nerve, e g on fracture of the temporal bone, the examinations mentioned may provide useful level diagnostic information However, the observations made in our two cases can only be explained by a partial lesion of the nerve fibres Part of the lesion—or even the whole lesion—may be localized central to the geniculate ganglion This calls for circumspection when trying to locate the lesion especially in cases of acute non traumatic peripheral facial palsy where the cause is not found and therefore the site of the lesion is unknown It does not seem justified to fix a limit as to how long centrally in the course of the nerve the lesion may be located based on an examination of tear secretion stapedial reflex and taste In a palsy affecting only the sense of taste the lesion may well be located central to the geniculate ganglion if the lesion of the facial nerve is partial and involves only some of the nerve fibres This must be borne in mind when deciding if the lesion is accessible to surgery It is paradoxical that Sir Charles—who founded his statements on well localized lesions of the facial nerve—should get his name attached to just those types of palsies where the site of lesion is unknown In cases of Bell s palsy the results of examinations of the tear secretion, the stapedial reflex and the sense of taste probably give no decide clue as to the site of the lesion but only an indication of the extent of the injury

ZUSAMMENFASSUNG

Bei akuter nichttraumatischer Gesichtslähmung mit unbekannter Ursache scheint es nicht gerechtfertigt Schlußfolgerungen darüber zu ziehen wie weit zum Zentrum im Verlaufe des Nerven die Läsion lokalisiert ist und zwar auf Grund einer Untersuchung von Tränensekretion Stapediusreflex und Geschmack Dies wird an zwei Fällen gezeigt

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CHANGES IN HUMAN NASAL RESISTANCE ASSOCIATED WITH EXERCISE HYPERVENTILATION AND REBREATHING

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Abstract The nasal resistance to airflow was determined in four subjects for periods of up to 7 hr Cyclic changes in the resistance of each nasal passage were demonstrated in 13 of 24 experiments After exercise on the cycle ergometer the total nasal resistance decreased and this change in nasal resistance was found to be directly related to the work rate After oral rebreathing the total nasal resistance decreased and after hyperventilation the total nasal resistance increased These changes in resistance are believed to be caused by changes in arterial pCO_2 and mediated by the autonomic innervation of the nasal vasculature

Cyclic changes in the resistance of each nasal passage have been previously described (Kayser, 1895) and the nasal cycle in man has been ... l documented by several authors using dif... methods (Heetderks, 1927, Beickert, ...1, Stoksted 1952, Principato & Ozen..., 1970)

The nasal cycle is due to the regular dilation and constriction of the venous cavernous tissue in the mucosa of the conchae and septum (Beickert, 1951 Bojsen Møller & Fahrenkrug, 1971) The changes are reciprocal with the conchae swollen on one side of the nose whilst conchae on the other side of the nasal cavity are constricted Cyclic changes in nasal resistance occur in 80% of humans and the cycle has a period of between 0 5 to 4 hr with a mean of 2 5 hr (Heetderks 1929) The reciprocal nature of the changes in nasal resistance on each side of the nasal cavity means that the total resistance to air flow remains relatively constant (Stoksted 1952)

With moderate exercise respiration occurs normally through the nose and is often accompanied by a subjective feeling of decreased nasal resistance Little research has been done on changes in nasal resistance during exercise An experimental study on the effect of exercise on nasal resistance in patients suffering from allergic rhinitis (Richerson & Seebohm 1968), demonstrated that a decrease in nasal resistance occurred during exercise yet these results are not applicable to normal individual as the patients had a diseased nasal mucosa and exhibited a very high nasal resistance under resting conditions

The present study is intended to demonstrate that changes in nasal resistance occur in normal subjects during exercise and also during periods of hyperventilation or rebreathing

METHODS

Subjects

Experiments were performed on 4 healthy males aged 20–27 None of the subjects had a history of allergic rhinitis, asthma or deviated septum, and none of them was prone to recurrent nasal or sinus infections

Rhinometry

Nasal air flow was measured with two electrospirometers (Mercury Electronics Ltd) with pneumotachograph flow heads connected

to polythene cannulae The cannulae were inserted into the infundibulum of each nostril Different sizes of matched pairs of cannulae were used to ensure a good seal between the nostril and cannulae in each subject The internal diameter of the cannulae was constant at 1 cm with a length of 12 cm

Posterior nares pressure was measured with a sensitive pressure transducer (S E Lab Ltd) A thick walled polythene cannula connected to the transducer was inserted into the mouth of the subject and manipulated until it lay well back on the tongue To prevent the soft palate touching the tongue and blocking the airway to the posterior nares the subject was instructed to bite gently on the cannula and so depress the tongue as to maintain patency with the nares With the cannula in position the subject sealed his lips around the tube to prevent any air from escaping through the mouth

Left and right nasal air flow and posterior nares pressure were recorded simultaneously on a pen recorder (Devices Ltd)

At the beginning and end of each experimental period both spirometers were calibrated using nitrogen flowing through a calibrated flow meter and pneumotachograph cone in series A water manometer was used to calibrate the pressure transducer

The resistance to air flow through each nasal passage was calculated from the formula

$$R = \frac{\Delta P}{V^2}$$

Where R is equivalent to the resistance $\Delta P=$ the pressure difference between anterior and posterior nares and V=air flow This formula has been shown to best fit the turbulent air flow in the nasal passage where during the great part of the breathing cycle there is a square law relationship between pressure and flow (Spoor 1965) The resistance units can be expressed in terms of pressure volume and time as cm H_2O (l/min) $^{-2}$

In the exercise experiments each resistance value was calculated as the mean resistance of ten consecutive inspirations In the rebreathing and hyperventilation experiments the mean of five consecutive inspirations was used for the calculations

Exercise

A 20 inch step was used for 5 min periods of severe exercise at the rate of 30 steps a minute A cycle ergometer was used for longer periods of exercise at set work rates The speed of pedalling was constant at 2 Hz and the load was adjusted to work rates between 30–180 Watts Subjects pedalled for three 10 min periods with 2 min rest intervals in between each exercise period for determination of nasal resistance

Measurements of nasal resistance were made every half hour for 3 hr before and after the exercise periods More frequent measurements were made during the half hour period after the exercise when measurements were made at 5 10 min intervals The subjects breathed freely through mouth or nose during the periods of exercise yet the mouth was closed during the short period when the nasal resistance was actually determined

The subjects were asked to rest during the pre and post exercise periods and to avoid any exertion excitement and changes in humidity or temperature During the experiments the subjects usually read in an office next to the laboratory The range of temperature in the laboratory was between 18–26°C and the relative humidity was between 30–45%

Hyperventilation and rebreathing

Rebreathing and hyperventilation experiments were performed either through the nose or mouth and measurements of nasal resistance were made every 30 sec for 20 min before and after the test period Test periods were separated by at least 2 hours to allow full recovery towards normal

*Subjects performed oral and nasal hyper*ventilation at maximal ventilatory capacity for 3 min This was found to be the

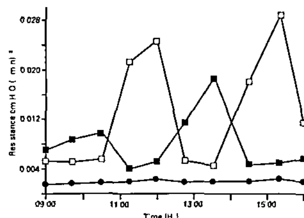


Fig 1 Nasal resistance of right □ and left ■ nasal passages and total nasal resistance ● in a normal resting subject showing a definite nasal cycle Each point represents the mean resistance value of ten consecutive *breaths*

length of time which could be tolerated without too much discomfort or syncope

Oral rebreathing was from a 10 l capacity bag filled with air and lasted until dyspnoea became uncomfortable Nasal rebreathing was performed with plastic cannulae inserted into the infundibulum of each nostril The cannulae formed a *y*-piece attached to the rebreathing bag, and the subjects held the *y*-piece in position to prevent displacement during the exertion of rebreathing

RESULTS

The rhinometric method developed for these experiments was found to be satisfactory in measuring the separate resistance of each nasal passage quickly and simultaneously

In 4 subjects measurements of nasal resistance were made every half hour for periods of up to 7 hours and cyclic changes in nasal resistance were found on 13 out of 24 occasions Fig 1 is a graphic representation of the results of one of these experiments in which a regular nasal cycle was observed Cyclic changes in nasal resistance did not normally begin until the subject had been in the laboratory atmosphere for about one hour and during this period total nasal resistance rose to a constant value which usually remained stead[y] throughout the day Some day to-day varia[-] tion was found in total resistance, e g t[he] mean total nasal resistance for 13 experimen[ts] on one subject was 0 0024±0 00039 (S D) c[m] $H_2O/(l/min)^{-2}$

On the occasions when a nasal cycle w[as] not apparent one nasal passage usually had low resistance which was constant, whilst t[he] other had a high resistance which had som[e] cyclic activity *In these cases total nasal r[e]*sistance varied more than was normal

Effect of exercise on nasal resistance

Preliminary experiments used stepping exe[r]cise, and a significant decrease in *total nas[al]* resistance was found in four tests on 2 su[b]jects The mean decrease was 0 00096 [±] 0 00030 (S D) cm $H_2O/(l/min)^{-2}$ from a me[an] total resistance of 0 0019 cm $H_2O/(l/min)$ ($p<0.01$) The decrease in total resistance wa[s] due to decreases in both left and right airwa[y] resistances Total resistance returned to i[ts] pre exercise level in a mean time of 30 min

Subsequent experiments involved a cyc[le] ergometer for exercise, and 4 subjects exe[r]cised at a known work rate for three consecu[-] tive 10 min periods Each subject was teste[d] at several different work rates between 3[0] [and] 180 W

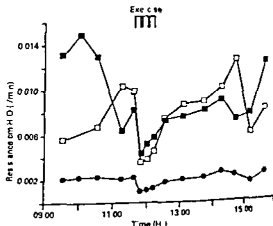


Fig 2 Changes in right □ ... ft ■ and total ● nasal r[e]sistance in one subject with ... riods of exercise at 1 ... y ... po nt represents the ... secutive breaths

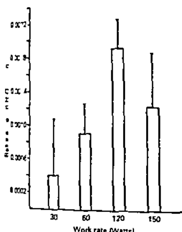


Fig 3 Histogram of change in nasal resistance with work rate The values represent the decrease in resistance observed in one subject and are represented with S D bars Each point represents the mean resistance value of ten breaths recorded after the last 10 min period of exercise

The subjects reported a decrease in nasal resistance soon after the start of the first exercise period and in a total of fifteen experiments a significant ($p<0\ 01$) decrease in total resistance was found for all 4 subjects at work rates above 30 W The effects of three 10 min periods of exercise at 180 W in one subject are shown in Fig 2 A decrease in total nasal resistance was apparent immediately after each exercise period and this was due to decreases in the resistances of both right and left airways The total nasal resistance rose slowly after the exercise periods and often took up to an hour to return towards the control measurements Cyclic changes of nasal resistance were usually apparent before and after the exercise periods but the effects of exercise appeared to disrupt the regular nasal cycle of reciprocal changes in resistance of left and right airways The decrease in total nasal resistance was linearly related to work rate for rates between (30–120 W) and significant regressions ($p<0\ 01$) were calculated for the 2 most studied subjects in a total of eight experiments In the same 2 subjects there was no further decrease in nasal resistance at work rates above 120 W The changes in nasal resistance in one subject as[illegible] with four different work rates are shown in Fig 3 The linear relationship between work rate and decrease in total resistance is apparent in this subject for work rates of 30, 60, and 120 W, yet at 150 W there is no further decrease in resistance in this subject

Rebreathing experiments

Three subjects were used for seven rebreathing experiments, each subject rebreathed air from a 10 litre bag until breaking point A significant ($p<0\ 01$) decrease in total nasal resistance was found in all seven experiments with a mean decrease of $0\ 00082 \pm 0\ 00030$ (S D) cm $H_2O/(l/min)^{-2}$ The effects of oral rebreathing on nasal resistance are shown graphically in Fig 4 This result is typical in that the change in total resistance is due mainly to a decrease in airway resistance in the nasal passage with the highest resistance, and that the total nasal resistance quickly returned towards control values after the period of rebreathing The mean recovery time for nasal resistance was $2\ 1 \pm 1\ 4$ min (S D) No significant difference was found between experiments in which subjects used oral or nasal rebreathing

Hyperventilation

In 3 subjects oral hyperventilation for periods of 3 min caused a significant increase in total

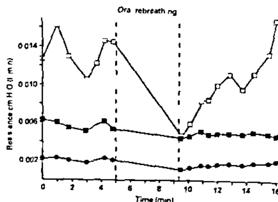


Fig 4 Changes in right □ left ■ and total ● nasal resistance in one subject associated with oral rebreathing Each point represents the mean resistance consecutive breaths

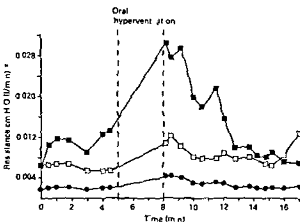


Fig 5 Changes in right □ left ■ and total ● nasal resistance in one subject associated with 3 minutes of oral hyperventilation Each point represents the mean resistance value of five consecutive breaths

nasal resistance in seven out of eight experiments The mean increase in total nasal resistance was 0 00093±0 00060 (S D) cm H_2O/$(l/min)^{-2}$ ($n=8$) The mean recovery time for nasal resistance to return to control values was 2 6±2 0 min (S D) The effect of hyperventilation on nasal resistance is illustrated graphically in Fig 5 which demonstrates that nasal resistance increased in both nasal passages and returned towards control values in approximately 5 min The same 3 subjects hyperventilated for 3 min through the nose in six experiments, and in five of the experiments no significant change in nasal resistance could be measured and a significant ($p<0.01$) increase was found in only one experiment

DISCUSSION

Cyclic changes in nasal resistance have been demonstrated in resting subjects and these results confirm those of previous authors who have demonstrated a nasal cycle in man (Heetderks, 1927, Stoksted, 1952 Principato & Ozenberger, 1970) The pheromenon of the nasal cycle has also been demonstrated in the rat and rabbit (Bojsen-Møller & Fahrenkrug, 1971), and in the domestic pig (Eccles & Maynard, 1975), yet the significance of the nasal cycle remains obscure Since the total nasal resistance remains relatively constant due to the reciprocal nature of the changes in resistance of each nasal passage there must be some central regulation of the nasal cycle The changes in nasal resistance may indicate that there are changes in activity of autonomic centres in the brain, as it has been previously demonstrated in man that there are cyclic changes in the size of each pupil in phase with the nasal cycle indicating that these changes in autonomic activity are not restricted solely to the nasal mucosa (Beickert 1951)

The results of this study demonstrate that a significant decrease in total nasal resistance occurs during stepping or cycling exercise The magnitude of the change in nasal resistance is directly related to the work load for work rates between 30–120 W. In a previous study (Aschan et al , 1958) this change in nasal resistance during exercise has been attributed to release of adrenalin from the adrenal medulla, yet this is unlikely as the change in nasal resistance is abolished during blockade of the stellate ganglion (Richerson & Seebohm 1968) An increase in sympathetic tone to the nasal mucosa is the most likely explanation for the response, yet the factors regulating the response are unknown The decrease in nasal resistance during exercise fits into the general respiratory response to exercise as it will facilitate rapid exchange of gas along the respiratory tract

The decrease in total nasal resistance during rebreathing is probably directly related to the rise in arterial pCO_2 and may be due to an increase in sympathetic tone to the nasal vasculature rather than a direct effect of carbon dioxide on the nasal blood vessels. In the do the nasal vasoco response associated with asphyxia h after section of (Tatum, 192 obtained o lished wo mals the pathetic

nasal resistance in response to asphyxia or rebreathing is consistent with the changes in nasal resistance observed in response to exercise in both instances the response to an increased requirement for ventilation of the lungs is a decrease in nasal resistance

An increase in total nasal resistance was observed in response to hyperventilation, and this may be directly related to a fall in arterial pCO_2 yet it is probably not regulated through the sympathetic nervous system as the increase in nasal resistance during hyperventilation in the dog was not affected by section of the cervical sympathetic nerve (Tatum, 1923) It has also been demonstrated in man that the increase in resistance of the lower respiratory tract during hyperventilation is greatly reduced after atropine, which indicates that the response may be mediated by the parasympathetic nervous system (Newhouse et al, 1964) Nasal hyperventilation in contrast to oral hyperventilation did not usually cause a significant increase in nasal resistance and this is probably because high rates of hyperventilation were difficult to achieve when breathing solely through the nose

The present study demonstrates that the autonomic nervous system regulates nasal resistance in response to exercise, rebreathing and hyperventilation and that the nasal mucosa has an active role in the respiratory response to these activities

ZUSAMMENFASSUNG

Bei vier Versuchspersonen wurde der Luftstromungswiderstand wahrend Untersuchungszeiten bis zu 7 Std gemessen Bei 13 von 24 Versuchen wurden zyklische Veranderungen des Widerstandes beider Nasengange nachgewiesen Nach Belastung auf dem Fahrrad Ergometer fiel der Gesamtnasenwiderstand ab und es konnte nachgewiesen werden daß diese Änderung des Nasenwiderstandes in direkter Beziehung zur geleisteten Arbeit stand Nach Ruckbeatmung aus einem geschlossenen System fiel der Gesamtnasenwiderstand ab und nach Hyperventilation stieg der Gesamtnasenwiderstand an Es wird angenommen daß diese Widerstandsanderungen durch Veranderungen des arteriellen pCO_2 hervorgerufen werden und daß sie durch die vegetative Innervation des Nasengefäßsystems vermittelt werden

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THE FREQUENCY AND IMPORTANCE OF BRONCHIAL HYPERREACTIVITY IN PATIENTS WITH ALLERGIC AND NON-ALLERGIC RHINITIS

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Abstract A trial was undertaken to evaluate the occurrence of bronchial hyperreactivity, typical of bronchial asthma in 50 patients with hay fever (rhinitis allergica) and 45 patients with rhinitis vasomotorica nonallergica as opposed to a group of healthy subjects and patients with bronchial asthma All the patients were subjected to spirographic examinations at rest (VC FEV_1 ETT SI) after exercise (PWC_{170}) and after histamine inhalation The authors believe that it is expedient to study bronchial hyperreactivity in patients with hay fever and rhinitis vasomotorica nonallergica in that it affords possibilities for the prevision of the conceivable unfavourable evolution of the disease towards the atopic or non atopic bronchial asthma as well as for the taking of adequate preventive and therapeutic measures

rom a review of the literatlure it follows that in patients with seasonal hay fever (rhinitis allergica), not specifically treated with allergenic pollen, bronchial asthma develops in 22–75% of patients (Broder et al , 1962) In the described groups of patients with hay fever, the pathological signs in the upper respiratory tract were accompanied in a high percentage of cases by simultaneous bronchial asthma symptoms of varying expression in Roux (1970) in 49 9%, in Serafini (1957) in a group of patients sensitized by Parietaria officinalis pollen in 67 5%, and in our examinations in 26 8% of adult subjects and in 40 5% of cases among children (Gniazdowski 1975 Martynowski & Gniazdowski, 1976) It is generally known that pollen allergy occurs in 10 5 to 40 0% of the examined asthmatics (Anderson 1974, Blair, 1974, Mantle & Pepys, 1974, Quellette & Reed, 1967) The problem of the evolution and coexistence of rhinitis vasomotorica nonallergica with bronchial asthma has not yet been thoroughly discussed in literature

It is nowadays fairly well known that a very common feature of all patients with bronchial asthma whether atopic or non atopic, is bronchial hyperreactivity, or the respiratory tract predisposition to spasms and myxiosis under the influence of various stimuli The above feature occurs in those persons who are susceptible to bronchial asthma already in the asymptomatic period, which facilitates with the aid of appropriate examinations, the recognition of latent predisposition to this disease (Romanski, 1976)

On the grounds of the above observations examinations were undertaken in order to evaluate the frequence of occurrence of bronchial hyperreactivity as a stigma of bronchial asthma in patients with hay fever and rhinitis vasomotorica nonallergica, in comparison with the frequence of occurrence of bronchial hyperreactivity observed in patients with bronchial asthma, as well as healthy subjects

MATERIAL

The examinations were carried out on the patients treated at our clinic and at the Clinic Otolaryngological Outpatient Department

Table I *Number of cases with outstanding (++++), very high (+++) or medium (++) bronchial hyperreactivity in persons with hay fever, rhinitis vasomotorica nonallergica, bronchial asthma and in healthy subjects*

n number of cases

Clinical form	PWC_{170} (++++) *n*	%	Histamine tests (+++) *n*	%	(++) *n*	%	Total *n*	%
Hay fever *n*=50	7	14 0	11	22 0	11	22 0	29	58 0
Rhinitis vasomotorica nonallergica *n*=45	1	2 2	6	13 3	3	6 7	10	22 2
Bronchial asthma *n*=30	18	60 0	10	33 3	2	6 7	30	100 0
Healthy subjects *n*=30	0	0 0	0	0 0	0	0 0	0	0 0

++++ Bronchoconstriction following PWC_{170} +++ and ++ bronchoconstriction following Histaminum dihydrochloricum inhalation of 0 125 or 0 25 mg/ml/3 min

I *Patients with hay fever*

The examinations were carried out on 50 patients aged between 19 and 50 (78 0% under 40) with a typical clinical picture of hay fever (no concomitant symptoms from the lower respiratory tract) supported by test and provocation investigations In 42 persons (84%) a relationship with heredity was discovered In the group of hay fever patients, affected for less than 10 years, there were 29 subjects (58%) whereas in the group of patients affected for more than 10 years there were 21 subjects (42%)

II *Patients with rhinitis vasomotorica nonallergica*

The examinations were carried out on 50 patients aged between 19 and 54, in whose cases the allergic etiology of the disease and the coexistence of symptoms from the lower respiratory tract were ruled out In 41 patients (91 2%) the clinical symptoms of rhinitis vasomotorica nonallergica lasted for about 10 years, and in 4 patients (8 8%) for over 10 years

III *Patients with bronchial asthma*

The examinations were performed on 30 patients with bronchial asthma, aged berween 19 and 51 None of the patients was given drugs, and they all had normal ventilation efficiency of lungs at rest The diagnosis of bronchial asthma was arrived at in this group of patients on the basis of clinical and allergological examinations The patients with bronchial asthma constituted the control group for the positive result of provocation tests

IV *Group of healthy subjects*

The examinations were performed on 30 healthy subjects aged between 19 and 53, in whose cases, on the basis of clinical and allergological examinations, no deviations in either the upper or the lower respiratory tract were discovered The healthy subjects constituted the control group for the negative result of provocation tests

METHOD

All the patients were subjected to scrutiny, including a detailed case history, otolaryngological examination, allergological examination, and provocation tests In the latter, for each of the four groups, there were carried out spirographic examinations at rest marking vital capacity (VC), forced expiratory volume in one second (FEV_1), expiratory total time (ETT), and spirographic index (SI) of Dubois de Montreynaud (Medicor expirograph), after controlled exercise of the PWC_{170} type (Monark cycle ergometer), and also

Table II *Number of cases with outstanding (++++) very high (+++) or medium (+ bronchial hyperreactivity in patients of age under 40 and over 40 with hay fever and rhin vasomotorica nonallergica*

n=number of cases

Clinical form of the disease	PWC$_{170}$ (++++)		Histamine (+++)		Histamine (++)		Total	
	n	%	n	%	n	%	n	%
Under 40 years of age								
Hay fever n=39	4	10 3	6	15 4	9	23 0	19	48 7
Rhinitis vasomotorica nonallergica n=36	1	2 7	6	16 7	2	5 6	9	25 0
Over 40 years of age								
Hay fever n=11	3	27 3	5	45 5	2	18 2	10	91 0
Rhinitis vasomotorica nonallergica n=9	0	0 0	0	0 0	1	11 1	1	11 1

inhalation in increasing doses (ultrasonic nebulizator TUR USI 3) with the aid of our own method (Dziedziczko, 1976) The results of the marked spirographic parameters were counted in decrease percentages, a FEV$_1$ value decrease greater than 10% being considered significant The bronchial hyperreactivity characteristic of bronchial asthma was marked outstanding (++++) very high (+++) and medium (++)

The provocation tests were carried out on hose patients who on the basis of clinical and lergological examinations did not show any vidence of bronchial asthma and the spirographic examinations indicated no bronchoconstriction

RESULTS

Positive results of the exercise and histamine provocation tests (taken together) in the group of patients with hay fever (Group I) were noted in 29 cases (58%) in patients with rhinitis vasomotorica nonallergica (Group II) in 10 cases (22 2%) whereas in the group of patients with bronchial asthma (III) they were noted in 100% of cases and among the healthy subjects (Group IV) no such results were noted

The exercise test (PWC$_{170}$) indicated disturbances in the ventilation of lungs in th spirographic examination (outstanding bronchial hyperreactivity ++++) in 7 patien (14%) of Group I, in 1 patient (2 3%) of Grou II, and in as many as 18 patients (60%) Group III

The spirographically perceptible FEV$_1$ a SI value decrease after a 3 min histamine i halation appeared in 22 patients (44%) Group I, in 9 patients (19 9%) of Group and in 12 patients (40%) of Group III (ve *high +++ or medium ++*, bronchial hype reactivity)

In patients under 40 the bronchial spas after exercise occurred in 4 cases of hay fev (10 3%) and in 1 case of rhinitis vasomoton nonallergica (2 7%) and after histamine i halation in 15 cases of hay fever (38 4%) a in 8 cases of rhinitis vasomotorica nonall gica (22 3%) (Table II) Among patients ov 40 the bronchial spasm after exercise occurr only in 3 cases of hay fever whereas aft histamine inhalation in 7 cases of hay fev (63 7%) and in 1 case of rhinitis vasomoton nonallergica (11 1%) (Table II)

Among subjects with the disease lasting le than 10 years, the bronchial spasm after exe cise occurred *in 2 cases of hay fever* (6 whereas after hi halat on in of hay fever (5

Table III *Number of cases with outstanding (++++) very high (+++) or medium (++) bronchial hyperreactivity in patients with evolution time of hay fever and rhinitis vasomotorica nonallergica lasting 10 years and above 10 years*

n number of cases

Clinical form of the disease	PWC170 (++++)		Histamine tests (+++)		Histamine tests (++)		Total	
	n	%	*n*	%	*n*	%	*n*	%
Evolution under 10 years								
Hay fever *n*=29	2	6 9	7	24 1	8	27 6	17	58 6
Rhinitis vasomotorica nonallergica *n*=41	0	0 0	6	14 6	3	7 3	9	22 0
Evolution over 10 years								
Hay fever *n*=21	5	23 8	4	19 1	3	14 3	12	57 1
Rhinitis vasomotorica nonallergica *n*=4	1	25 0	0	0 0	0	0 0	1	25 0

vasomotorica nonallergica (22 0%) (Table III) Among subjects with the disease lasting more than 10 years, the bronchial spasm after exercise occurred in 5 cases of hay fever (23 8%) and in 1 case of rhinitis vasomotorica nonallergica (25 0%), and after histamine inhalation in only 7 cases of hay fever (33 4%) (Table III)

DISCUSSION

Bronchial hyperreactivity to various exogenic and endogenic factors such as inhalatory allergens, infections, climatic changes air pollution nervous and hormonal factors and physical exercise, occurs as a characteristic feature in persons affected by bronchial asthma In laboratory conditions it is possible to reveal it in every patient by means of exercise and histamine tests (Anderson et al 1975 Curry 1946, Dziedziczko, 1975, Makino 1966)

The examinations of reactivity of the bronchi in patients with hay fever carried out, up till now, by other authors have given contradictory results First Curry (1947), and then Feinberg et al (1952) stated that histamine provocation tests performed on patients with hay fever did not indicate spirographically perceptible changes in the examined parameters However, such changes were discovered in some patients with hay fever b n et al (1946) and Quellette & Reed (1967) Saroja et al (1975) revealed that in a certain number of patients with hay fever, under the influence of dosed physical exercise, there appeared a significant decrease in the marked spirographic indicators

Our examinations showed that the bronchial hyperreactivity to exercise and histamine, typical of bronchial asthma, occurred in patients with hay fever in 58 0% of cases, whereas in patients with rhinitis vasomotorica nonallergica in 22 2% of cases In each examined case of bronchial asthma, under the same exercise and histamine provocation conditions, bronchial spasm appeared whereas among healthy subjects, no spastic changes of the bronchi were noted after the provocation tests In patients with hay fever and rhinitis vasomotorica nonallergica, in comparison with patients with bronchial asthma, the percentage of cases of outstanding bronchial hyperreactivity (++++) was significantly lower (Table I)

The results of our examinations also indicated that the initially lower percentage of patients with outstanding bronchial hyperreactivity becomes significantly higher after 40 years of age and when hay fever has lasted for 10 years, on the other hand, the percentage of those patients in whose cases the bro hyperreactivity was found to

creases (Tables II and III) This would seem to prove that in patients with hay fever, and presumably also in patients with rhinitis vasomotorica nonallergica, the degree of bronchial hyperreactivity may increase

The differences in the degree of intensification of bronchial hyperreactivity observed among patients with hay fever and rhinitis vasomotorica nonallergica suggests that, although many of them reveal susceptibility to bronchial asthma, it is not the same with each of them, and, in some cases, it may develop in the course of the disease along with age, under the influence of certain unfavourable environment conditions It also seems that in patients in whose hay fever and rhinitis vasomotorica nonallergica is associated with significant bronchial hyperreactivity, one has to look for their common organic basis

Hay fever is one of the classical atopic diseases The bronchial hyperreactivity discovered in our patients with hay fever (in 58 0% of the examined cases) must also be conditioned hereditorily It has been revealed that the mucous membrane of the respiratory tract responds with an immediate allergic reaction as a whole (Romanski et al , 1976, Romanski, 1976) Therefore, the bronchial hyper-...ity accompanying hay fever makes the ... susceptible to future atopic bronchial ...thma, and it also explains numerous cases where these two pathological complexes associate

Rhinitis vasomotorica nonallergica does not associate with atopy symptoms Hence, the bronchial hyperreactivity occurring in the exercise and histamine provocation tests in 22 5% of the patients does not result from the hereditorily conditioned predisposition to allergic diseases It is then possible to conclude that the bronchial hyperreactivity observed in patients with rhinitis vasomotorica nonallergica may be a sign of their predisposition to being affected by non atopic bronchial asthma in the future

There are still many unexplained factors in the pathomechanism of hay fever rhinitis vasomotorica nonallergica, and bronchia[l] hyperreactivity, both atopic and non atopic The propounded theories do not account fo[r] many mechanisms (Arthur & Shelley, 1938 Mason, 1967, Reed, 1974, Simmonson et al 1967) One may believe, though, that bronchia[l] hyperreactivity is a common feature of al[l] cases of bronchial asthma and that it never oc[-] curs in completely healthy persons There[-] fore, the discovery of the occurrence of bron[-] chial hyperreactivity in 58 0% of patients wit[h] hay fever and in 22 5% of patients with rhinitis vasomotorica nonallergica must be considere[d] to be those patients' special predisposition t[o] being affected with atopic and non atopi[c] bronchial asthma The discovery of bronchia[l] hyperreactivity in the examined patient is, n[o] doubt, tantamount to the assertion of the pre[-] disposition to bronchial asthma The degree o[f] imminence of this disease can be additionall[y] set by marking off of the outstanding (++++), high (+++), and medium (++) bronchia[l] hyperreactivity

It seems, then, that investigating bronchia[l] hyperreactivity in patients with hay fever an[d] rhinitis vasomotorica nonallergica by means o[f] simple provocation tests (exercise and his[-] tamine) is reasonable, since it renders possible the prevision of the conceivable unfavourable evolution of the disease towards atopic and non atopic bronchial asthma, thus enabling one to take preventive and therapeutic meas[-] ures

CONCLUSIONS

1 The exercise and histamine provocatio[n] tests made possible the diagnosis of the bron[-] chial hyperreactivity typical of bronchial asth[-] ma in 58 0% of patients with hay fever, i[n] 22 2% of patients with rhinitis vasomotoric[a] non allergica, and in 100% of patients wit[h] bronchial asthma, however, no evidence o[f] bronchial hyperreactivity was found in any o[f] the 30 healthy subjects

2 The frequency of bronchial hyperreac[-] tivity and its degree of intensity increase in pa[-] tients with hay fever and, presumably, also i[n]

patients with rhinitis vasomotorica non-allergica along with age and evolution of the disease

3 The occurrence of the bronchial hyperreactivity characteristic of bronchial asthma in patients with hay fever and rhinitis vasomotorica nonallergica attests to the possible existence of the atopy in the former case, and in the latter case, non atopy basis for the respiratory tract s hyperreactivity, which means predisposition of those patients to being affected with atopic and non atopic bronchial asthma in the future

4 Early diagnosis of bronchial hyperreactivity in patients with hay fever and rhinitis vasomotorica nonallergica on the grounds of the results of exercise and histamine provocation tests enables one to foresee the con ceivable unfavourable evolution of the dis ease, and to take appropriate preventive and therapeutic measures with these patients

ZUSAMMENFASSUNG

Man hat die Probe unternommen die Häufigkeit des Auftretens der für das Bronchialasthma typischen Bronchialüberempfindlichkeit bei 50 Kranken mit Pollinosis (Rhinitis allergica) und 45 Kranken mit Rhinitis vasomotorica nonallergica im Vergleich zu einer Gruppe von gesunden Personen sowie den Bronchialasthma Kranken zu bestimmen Bei allen Patienten wurden spirographische Untersuchungen im Ruhezustand (VC FEV_1 ETT SI) nach einer Anstrengung (PWC_{170}) und nach der Einatmung des H stamine durchgefuhrt Die herausragende (++++) sehr große (+++) und mittelmaßige (++) Bronchialuberempfindlichkeit wurde bei 29 Kranken mit Pollinosis (58 0%) bei 10 Kranken mit Rhinitis vasomotorica nonallergica (22 0%) und bei allen Bronchialasthma Kranken (100 0%) aber bei keiner gesunden Person festgestellt Die A [illegible] Un ers chung der [illegible]

wicklung der Erkrankung zu atopischem und nicht atopischem Bronchialasthma und damit die rechtzeitige Unternehmung von geeigneten Vorbeugungs und Heilmitteln ermoglicht

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NUCLEAR BODIES IN LARYNGEAL PAPILLOMA

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Abstract Electron microscopy of Juvenile Laryngeal Papilloma from Nigerian children has revealed the presence of nuclear bodies in the neoplastic cells These bodies were round or oval in shape and varied from 0 3 to 0 5 μm in diameter The immature forms consisted of fine fibrous units whereas the mature forms displayed central and peripheral zones The central zone consisted of dense coarse granular filaments whereas the peripheral zone showed fine filaments of low electron density Some intermediate forms were also noted These bodies were similar to those reported in other viral infections and in tumour cells

The presence of spherical nuclear bodies in pathological material—especially in tumours and diseases of viral etiology—has been reported frequently (Robertson, 1964, Robertson & Maclean, 1965, Krishan et al 1966, 1967, Bouteille et al , 1967, Brooks & Siegel, 1967, Henry & Petts, 1969) However, the occurrence of such bodies in laryngeal papilloma has not been recorded It is the purpose of this report to present evidence and to describe the nuclear bodies which are seen in juvenile laryngeal papillomas taken from Nigerian children at fine structural level and also to discuss their probable significance

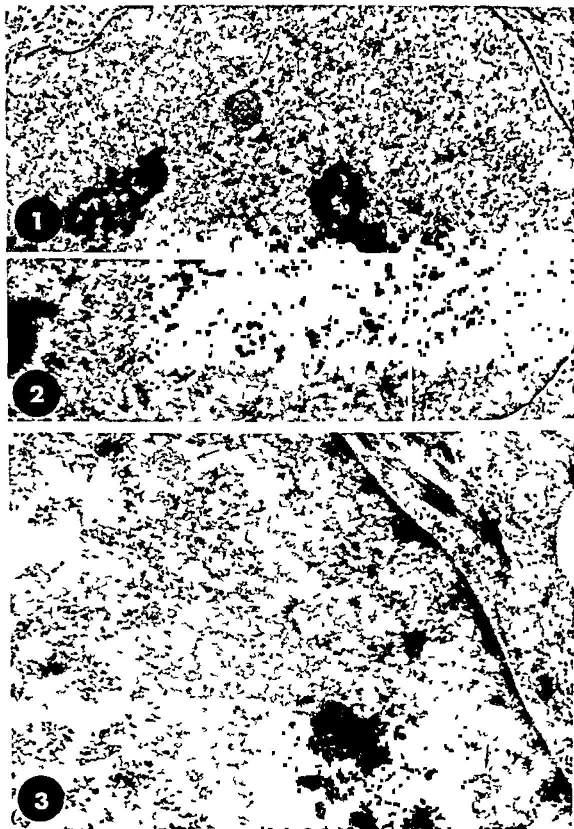
MATERIALS AND METHODS

The materials used for this study were surgical biopsy specimens taken from 5 Nigerian children suffering from multiple laryngeal papillomas Small pieces of papillomatous tissue were immersed in 3 [illegible] utaraldehyde at pH 7 4 in sodium cacodylate buffer After one hour post fixation in 1% osmic acid they were dehydrated in alcohol and embedded in Araldite Thin sections were cut with a Porter Blum ultra microtome, stained with uranyl acetate and Reynold s lead citrate and were examined with a Hitachi HS-8 electron microscope

RESULTS

In all 5 cases examined, we observed nuclear bodies of round or oval appearance in the tumour cells (Fig 1) At the fine structural level these bodies indicate a close morphological relationship in their size and shape to the nuclear bodies reported by Bouteille et al (1967) in neoplastic and virus infected cells Their size varied from 0 3 to 0 5 μm in diameter (Fig 2) Usually there were two spheroids in a single nucleus (Fig 1), but up to four such bodies were also seen in a section profile (Fig 3) In most of the sections the nuclear body was surrounded by a clear halo, relatively devoid of stainable nuclear material (Figs 1 and 2) There was no limiting membrane enclosing these bodies but they were well separated from the surrounding nucleoplasm

Although there were some variations in the appearance of nuclear bodies—from [illegible] mass of fibrous tissue, to concent[illegible] ranged fibrils—their basic stru[illegible]


1
2
3

me In the immature form, each body was mposed of fibrous material of fine nature ig 3), whereas in the mature form it showed ntral and peripheral zones (Figs 2 and 4) ie central zone varied in size from 0 16 to 35 μm in diameter and was made up of ectron-dense, coarse granular filaments The ripheral cortical zone showed fine filaments low electron density and the whole body owed an onion like architecture However, me intermediate forms were revealed, consisting of partially organized fine fibrous material together with a granular component (Fig

Occasionally, a large 'complex nuclear dy was also seen (Fig 5), which could have en a union of two, or an incomplete separation of two joined nuclear bodies

DISCUSSION

nce the first description by De The et al, 960), nuclear bodies have been reported in lls from a variety of neoplastic tissue and her pathological conditions of known viral igin (Bouteille et al, 1967, Krishan et al, 66, 1967, Robertson, 1964) These bodies ere only found to a limited extent in normal ssue (Buttner & Horstmann, 1967), whereas tumour cells they were not only seen frequently but also in large numbers Bouteille al, (1967) have classified the nuclear bodies to five groups according to their morphogical features They were observed in 22 different types of tumours, among which eight ases were of known viral origin (Bouteille et al, 1967) The nuclear bodies seen in the present study show somewhat similar morphological features to those seen by the above authors in cases of proved or supposed viral etiology, such as Leukoencephalitis and Necrotizing Encephalitis, as they contained central and peripheral zones

The significance and the function of nuclear bodies in tumour cells is not known However, they are associated with an increased activity of the cell (Bouteille et al, 1967, Dumont & Robert, 1971) and also with an altered metabolic state of the nucleus (Krishan et al, 1967) In the light of the above reports and since the nuclear bodies are regularly seen in neoplastic and in the virus infected cells (Granboulan et al, 1963, Caputo & Bellone, 1966, Ulrich & Kidd, 1966, Bouteille et al, 1967, Perier et al, 1967, Popoff & Stewart, 1968) one could hypothesize that these bodies in tumour cells may be related to the causative factors of neoplasm

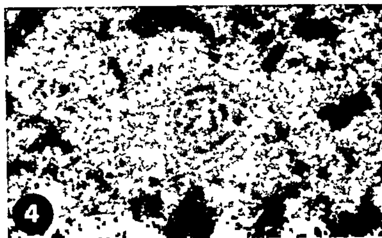
The exact etiology of Laryngeal Papilloma is not known, although the virus has been repeatedly postulated as being a causative agent (Ulmann, 1923, Dmochowski et al, 1964, Boyle et al, 1971, Ahmed & Mukherjee, 1974, Lundquist et al, 1975) It has also been suggested that there is some relationship between the nuclear bodies and viruses (Riviere et al, 1960, Granboulan et al, 1963, Bouteille et al, 1967, Perier et al, 1967, Popoff & Stewart 1968) The frequent occurrence of nuclear bodies in the present study provides further support for the suggestions made by above authors However, the present observations do not permit any conclusions to be drawn concerning the exact relationship of nuclear bodies with papilloma virus, other than that it may be associated with some stage of virus formation Therefore, further studies at morphological, tissue culture and histochemical levels are necessary in order to establish (*a*) if indeed such a relationship does exist between the nuclear bodies and the papilloma virus, (*b*) how the nuclear bodies are

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g 1 Low power micrograph of the tumour cell nucleus ith two nuclear bodies (↑) randomly scattered in the icleoplasm ×20 000

g 2 High power micrograph of a nuclear body of ature form from Fig 1 The nuclear body displays the iaracteristic pattern of inner and outer zone with coarse aments in the central core and fine microfibrillar ar ngements in the outer zone Note a clear zone separat g the nuclear body from the adjacent nucleoplasm 40 000

ig 3 Four nuclear bodies in a single nucleus The ar ows indicate nuclear bodies of immature form consisting fine fibrous material Note bodies of intermediate form the central part of the micrograph



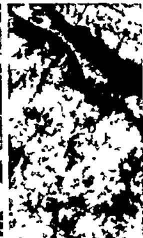


Fig 4 High power micrograph of the mature form of a nuclear body The fibrillar cortical zone surrounds dense and coarse filaments of central zone ×45 000

Fig 5 A complex nuclear body in the tumour c nucleus Note the collection of cytoplasmic fibrils f close to the nuclear membrane ×45 000

ACKNOWLEDGEMENTS

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ZUSAMMENFASSUNG

Elektronenmikroskopie von juveniler laryngealer Papilloma von nigerischen Kindern hat die Anwesenheit von nuklearen Korpern in den neoplastischen Zellen offenbart. Dieser Körper waren rund oder oval und variierten im Durchschnitt zwischen 0,3 und 0 5 µm Die unreifen Formen bestanden aus feinen faserigen Einheiten während die reifen Formen Zentral und Randzonen aufwiesen Die Zentralzonen bestanden aus groben korn gen Fäden während die Randzonen feine Fäden von niedriger Elektronendichte zeigten Einige Zwischenformen wurden auch beobachtet Diese Korper waren ahnlich jenen die in anderen Virusinfektionen und in Tumorzellen beschrieben worden sind

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ACIDIC GLYCOSAMINOGLYCANS IN THE GROUND SUBSTANCE IN PARANASAL SINUS DISEASES

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Abstract Acidic glycosaminoglycans (AGAG) in the normal maxillary sinus mucosae were analysed by electrophoretic separation and densitometric quantitation and then compared with those in chronic maxillary sinusitis nasal polyps and maxillary sinus cancer The total amount of AGAG in the edematous maxillary sinusitis and nasal polyps increased significantly Both increases were almost twice as large as the normal content When the changes of each AGAG in the total amount were compared with the normal content chondroitin sulfate A and C showed the most conspicuous increase chondroitin sulfate B was rather stable and hyaluronic acid showed a roughly twofold increase in all the tissues examined The ratio of %hyaluronic acid divided by %chondroitin sulfate B increased the most in maxillary sinus cancer

Chronic pathological changes around the paranasal sinuses, such as chronic sinusitis, polyp and maxillary sinus cancer, may affect the amount and characteristics of the connective tissue The connective tissue consists of three large components, the cells collagen fibers and ground substance The ground substance in the connective tissue includes important acidic glycosaminoglycans (AGAG or acid mucopolysaccharides) which act as an ion exchanger to control electrolytes and water in extracellular fluid and carry metabolites in the opposite direction by their acidity

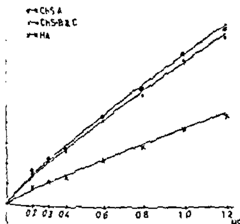
Therefore, AGAG in the normal maxillary sinus mucosae were analysed and compared with those in chronic paranasal sinusitis nasal polyps and maxillary sinus cancer

MATERIALS AND METHODS

Materials were obtained at operations Th sues analysed were human maxillary sinus cosae of normal and three types of chı maxillary sinusitis (edematous suppur and fibrous), nasal polyps and maxillary cancer None of them received previous t ment The normal maxillary sinus muc were obtained at operation of maxillary a ligation for nosebleed, or of maxillary frac The types of maxillary sinus mucosae determined by macroscopic and micros findings The mixed types were excluded this experiment

Extraction of acidic glycosaminoglycans from tissue

The tissue was cut into small pieces placed into acetone for over 3 days to re water and fat The acetone was changed eral times After removing the excess ace the tissue was placed in a desiccator t for over 7 days After measuring dry w (D W), the tissue was homogenized in of 0 01 M Tris HCl (pH 7 5) After addir ml of 5% pronase, the homogenized tissu incubated for 6 hr at 45°C To remove pr 1 8 ml of 22 2% TCA solution was then a After letting it stand for over 1 hr at temperature, it was centrifuged To the s



1 Calibration curves of authentic ChS A B C and obtained from the average (from 9 to 15 for each) of densitometer readings of the electrophoretograms.

...ant, absolute ethanol and potassium acetate were added to precipitate crude AGAG (Meyer et al., 1956) After chilling this solution overnight the precipitation was dissolved in distilled water and was then dialysed against distilled water To the dialysate, absolute ethanol and potassium acetate were again added to precipitate pure AGAG

Improved rapid quantitation of AGAG by electrophoretic separation reported by Seno et al. (1970) was used The precipitation, containing pure AGAG, was dissolved in an exact 1 ml of distilled water The extracted solution was applied on cellulose acetate strips soaked in 0 2 M calcium acetate buffer, from 4-8 µl as a 10 mm line at the origin (1 cm from the cathode end) After electrophoresis (1 mA/cm for 4 hr) the strips were stained with 0 5% Alcian blue in 3% acetic acid for 20 min, followed by washing with tap water for 10 min The strips were subsequently dried

The identification of AGAG was made by the mobility and enzymic digestion with testicular hyaluronidase (t HAase, Mochida & Takeda Pharmaceutical Co, *Tokyo) of 500* TRU in 1 ml of 0 2 M acetate buffer pH 5 2 for 24 hr at 37°C and chondroitinase ABC (CHase ABC Seikagaku Kogyo Co, *Tokyo)* of 5 units in 75 ml of 0 56 M Tris HCl buffer at pH 8 0 with 20 mg of bovine serum albumin for 1 hr at 37°C

The dried electrophoretogram was cleared in decalin, and the position and relative density of AGAG were scanned at 600 nm by a densitometer, Densitron SP-3 (Jokoo Sangyo Co, Tokyo), using a 0.5×7 mm slit width, at speed 3 and gain 5 AGAG were separated in the order of chondroitin sulfate C (ChS C), chondroitin sulfate A (ChS A), chondroitin sulfate B (ChS B) and hyaluronic acid (HA) from the anode Heparin (HP) and keratan sulfate (KS) migrated between ChS A and ChS B, while heparan sulfate (HS) migrated between ChS B and HA

Calibration curves of each AGAG were obtained from the densitometer reading by measuring the area under the curves A linear relationship between the amount of AGAG and the area under the curves of densitometer tracing was obtained for ChS A, B, C and HA

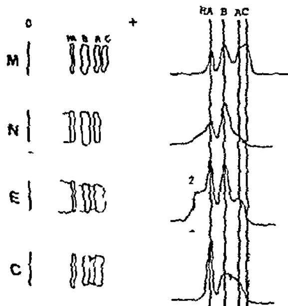


Fig 2 Electrophoretograms (*left*) and their densitometer tracings (*right*) Each vertical line on the densitometer tracings shows each position of AGAG Tailings and slow component were observed *M* authentic mixture of hyaluronic acid (*HA*) chondroitin sulfate B (*B*) chondroitin sulfate A (*A*) and chondroitin sulfate C (*C*) *N* normal maxillary sinus mucosa *E* edematous type of chronic maxillary sinusitis *C* maxillary sinus cancer

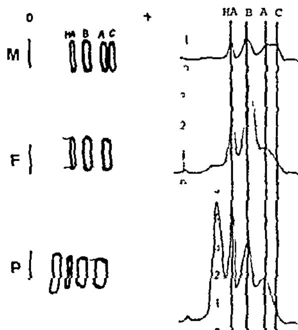


Fig 3 Electrophoretograms (*left*) and their densitometer tracings (*right*) The slow component in nasal polyp (*P*) is conspicuous *M* authentic mixture, *F*, fibrous type of chronic maxillary sinusitis

after the electrophoresis in 0 2 M calcium acetate buffer, in the concentration range of 0 2–1 2 μg (Fig 1) However, not all AGAGs showed the same density The percentage among AGAG content was therefore corrected from the calibration curve

RESULTS

(1) *Qualitative analysis of acidic glycosaminoglycans* (Figs 2, 3, 4)

The kinds of AGAG detected by electro retogram and enzymic digestion were Ch B, C and HA in all the tissues exami Among them, ChS-A, B and HA were the r components (Figs 2, 3, 4) Although the s ration of ChS-A and ChS C was not so c as shown in the figures ChS A was m greater in content than ChS C Thus, ChS-A and ChS C were calculated toget and were expressed as ChS-A(+C) Ot AGAG such as HS and HP were not detec by this method However, an unknown sl component and slight tailings between AGAG were observed The former which often detected in nasal polyps seemed to glycopeptides (Nakada et al , 1975), while latter seemed to be concerned with the pur and the position of the sulfate groups AGAG (Seno et al , 1970)

(2) *Quantitative analysis of acidic glycosaminoglycans* (Table I, Figs 5, 6)

The amount of AGAG per D W was 0 1 0 03 % (mean ± standard error, n=4) in t normal maxillary sinus mucosa, 0 32±0 05

le I *Analytical data of acidic glycosaminoglycans*

Tissues	Analytical items: No of examined	D W (mg) Mean	AGAG (mg) extracted Mean	%AGAG/D W Mean ± S E	%ChS-A(Mean ± S
Normal maxillary sinus mucosa	4	69 0	0 097	0 15±0 03	7±2 5
Edematous maxillary sinusitis	4	65 4	0 190	*↑ 0 32±0 05	*↑ 16±2 5
Suppurative maxillary sinusitis	4	49 2	0 165	NS 0 31±0 08	***↑ 24±3 6
Fibrous maxillary sinusitis	4	76 6	0 162	NS 0 24±0 04	NS 15±2 6
Nasal polyp	8	57 6	0 184	**↑ 0 32±0 04	*****↑ 26±2 4
Maxillary sinus cancer	7	58 8	0 125	NS 0 23±0 05	***↑ 26±3 9

Asterisks denote statistical significance * $P<0.05$ ** $P<0.02$ *** $P<0.01$, **** $P<0.005$ ***** $P<0.001$ NS significant ↑, increase, ↓ decrease

ı=4) in the edematous type of chronic maxil-
ry sinusitis, 0 31±0 08% (n=4) in the sup-
urative type of chronic maxillary sinusitis,
.34±0 04% (n=4) in the fibrous type of
ronic maxillary sinusitis, 0 32±0 04% (n=8)
ı nasal polyp, and 0 23±0 05% (n=7) in
axillary sinus cancer, respectively (Table I,
ig 5) AGAG in the edematous type of
hronic maxillary sinusitis and the nasal polyp
creased significantly Both increases were
most twice as large as the normal content
The normal maxillary sinus mucosa con-
ained 48±4 3% (mean ± standard error) of
A 45±2 3% of ChS B and 7±2 5% of ChS-
(+C) When the changes of each AGAG in
e total amount per D W were compared
ith the normal content as 100%, ChS-A(+C)
owed a most conspicuous increase, from 3
7 times as large as the normal content On
e other hand, ChS B was fairly stable, show-
g a slight increase in the edematous and
brous types of chronic sinusitis, and a slight
crease in maxillary sinus cancer (Fig 6)

) *Acidic glycosaminoglycans index*
Table I, Fig 7)

hen the ratio of %HA divided by %ChS B
AGAG index) was calculated, it showed 1 07

S-B ±S E	%HA Mean ± S E	AGAG index (%HA/%ChS-B) Mean ± S E
13	48±4 3	1 07±0 07
**↓ 17	NS 48±1 9	1 33±0 07
*↓ 6	NS 53±2 6	2 30±0 18
NS 13	NS 47±4 3	1 24±0 11
*↓ 28	NS 46±2 3	1 64±0 13
**↓ 0	NS 56±3 6	3 11±0 28

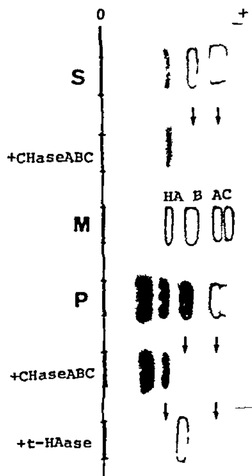


Fig 4 Electrophoretograms and their enzymic digestions The arrows show disappearances and decreases of each AGAG after enzymic digestions *S* suppurative type of chronic maxillary sinusitis *M* authentic mixture *P* nasal polyp *+CHaseABC* digestion with chondroitinase ABC *+t HAase* digestion with testicular hyaluronidase

in the normal maxillary sinus mucosa, 1 24 in the fibrous type, 1 33 in the edematous type, 1 64 in nasal polyp, 2 30 in the suppurative type and 3 11 in maxillary sinus cancer, respectively The greater the index number shows, the worse the condition of the mucous membrane becomes (Fig 7)

DISCUSSION

Electrophoretic separation of authentic
and ChS C in 0 2 M calcium acetat

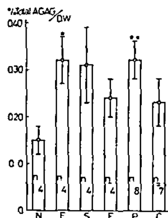


Fig 5 Total amount of AGAG per dry weight in the various tissues (mean ± S E) Asterisks mean statistically significant * $P<0.05$ * $P<0.02$

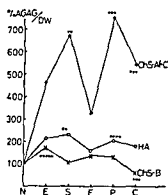


Fig 6 Changes of each AGAG per dry weight in the total amount compared with the normal as 100% * $P<0.05$ ** $P<0.02$ *** $P<0.01$ **** $P<0.005$ ***** $P<0.001$

pretty good, as reported by Seno et al (1970), although the electrophoretic mobility of both AGAG was very similar Therefore, the duration of electrophoresis was changed to 4 hr instead of the original 3 hr However, the actual separation of ChS A and ChS C from the extracted AGAG was not clear, but tailings were observed as shown in the figures (Figs 2, 3, 4) Therefore, both ChS A and ChS C were together estimated as ChS A(+C), because ChS A was much greater than ChS C in all the tissues examined Clear separation quantitation of the both AGAG remain to solved in the future

Jackson & Arihood (1971) extracted AGAG from human nasal polyps by NaCl solubility This was in agreement with our results that the largest component in nasal polyps was chondroitin sulfate, though HP was not detected in our experiment This disagreement with our results may be due to the sensitivity of the method employed The total amount of AGAG in nasal polyps reported by Jackson was 0 29 ±0 03% (S E), while our results showed 0 32 ±0 04% They were in good agreement

In the course of acute inflammation the amounts of hyaluronate and sialic acid increase, followed by the increments of ChS A and ChS-C, in the early stage ChS B then increases, and the total amount of AGAG returns to a normal level thereafter (Delaunary & Bazin, 1964) Wood (1960) reported that low concentration of ChS A and C accelerate collagen fibril formation in vitro On the other hand, ChS B is associated with the mature collagen (Loewi & Meyer, 1958, Koizumi et al 1967) From these points of view, the edematous and suppurative types of chronic sinusitis and polyps are related to the relative early stage of inflammation, while the fibrous type is related to the late stage However, high concentrations of ChS A and ChS C may interfere with the formation of collagen fibril, thus even the fibrous type still seems to remain as chronic inflammatory stage, showing a slight increase of ChS B and other AGAG One of the pathogens of chronic sinusitis and polyps therefore, was found to be the disturbance of connective tissue metabolism

HA is noted for its ability to bind wa (Ogston, 1966) The increase of HA found i edematous and suppurative sinusitis and i polyps, means higher water content in them

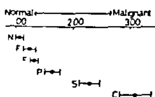


Fig 7 AGAG index which means the ratio of %HA ~ChS B

Maxillary sinus cancer showed increases of ChS-A(+C) and HA, and a decrease of ChS … AGAG index, which means a relative increase of HA, of maxillary sinus cancer was … the highest value among the other tissues. These increases of AGAG are due to the increased production of AGAG from both the cancer cells and connective tissue (Meyer & Chaffee 1940, Takeuchi, 1966). Increased AGAG is favorable to the growth of cancer cells (Takeuchi, 1966). The transformation of normal to malignant tumor tissues is also accompanied by an increase in negative electrical charge carried by the cells, and this negative charge increases as the cells acquire more malignant properties (Ambrose et al, 1956, Purdom et al, 1958). This negative charge derived from AGAG (Suzuki et al, 1970) correlated with a reduction in the adhesiveness of the tumor cells and with the appearance of early metastases (Ambrose et al, 1956 Purdom et al, 1958)

ZUSAMMENFASSUNG

Die elektrophoretischen qualitativen und densimetrischen quantitativen Analysen der sauren Glykosaminoglykane (SGAG) wurden in den normalen Oberkieferhohlen Schleimhauten verglichen mit der chronischen Sinusitis, den Nasenpolypen und den Oberkieferkrebsen aus geführt. Der totale Gehalt der SGAG bei chronischer ödematöser Sinusitis und Nasenpolypen zeigte bedeutende Zunahme die ungefähr zweifach so groß wie d e … der

[illegible]

erheblichste Zunahme Chondroitinsulfat B war … Zu stabil und Hyaluronsäure zeigte ungefähr zweifache Zunahme in allen untersuchten Geweben. Das Verhaltnis der Hyaluronsäure zum Chondroitinsulfat B nahm am meisten im Oberkieferkrebs zu

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MAXILLARY SINUSITIS

Effects of Treatment on the Local Antibacterial Defence

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Abstracts Low levels of immunoglobulins and complement in purulent antral secretion might jeopardize the local antibacterial defence To evaluate whether or not antibiotic treatment and antral drainage influence the local content of immunoglobulins and complement, samples of secretion were analysed both prior to drainage and 1–2 days after drainage In those patients requiring repeated drainage for their recovery, the local concentration of IgA and IgG was significantly lower in the secretion obtained prior to drainage than the local concentration found in patients who recovered after the initial drainage It was also demonstrated that after drainage, the level of IgA, IgG and the complement factors C3 and C4 increased significantly, whereas the proteolytic activity in the secretion usually decreased

Virulent microorganisms have not only the capacity to replicate in the biochemically complex environment of the host surface, fluid and tissue, but also the ability to resist the host defence, of which immunoglobulins and complement are important components

In a previous study, immunoglobulins were demonstrated in maxillary sinus secretion, the concentration being lower in purulent than in serous secretions (Carenfelt et al , 1976) As pus and certain purulent secretions have a high proteolytic activity (Lieberman & Kurnick, 1962) and as immunoglobulins and complement are susceptible to degradation by proteases, it was surmised that low levels of immunoglobulins and complement in purulent sinus secretion might result from proteolytic degradation

In the present study, the immunoglobulins IgA, IgG and IgM, the complement factors C3 and C4, and the protease inhibitor α_2-macroglobulin were determined in purulent sinus secretion in order to evaluate whether the treatment (including drainage of the purulent secretion) might influence the content of these components in the sinus secretion

This study was supported by grants from Sigurd and Elsa Golje Memorial Foundation

MATERIAL AND METHODS

Patients

Twenty-nine patients (21 female and 8 male, age rang 22–65 years) with symptoms of maxillary sinusitis for days or less and with a retained purulent secretion *sinus maxillaris* were selected for the present study [illegible]

secretion was then evacuated as completely as pos[illegible] resulting in volumes varying between 1 and 12 ml Th[illegible] antral drainage was repeated at intervals of 1–2 days [illegible] til the patient had recovered, that is until air or a [illegible] volume (<1 ml) of non-purulent secretion was obtaine[illegible] Irrigation was not used The therapy also included pe[illegible] cillin-V or doxycycline orally administered in ordina[illegible] dosis, together with decongestive drugs The antibio[illegible] treatment was usually initiated after the first drainage

Samples of secretion macroscopically tinged with blo[illegible] were discarded, excepting those samples obtained on [illegible] second or third aspiration (5 of 16 secretions) Samp[illegible] of venous blood for serum were taken simultaneous[illegible] The secretion samples and sera were stored at −2[illegible] until analysed immunologically

ʿeI ***Mean immunoglobulin concentration ,ml) in serum and purulent sinus secretion ...ned prior to drainage from 29 patients***

...eated aspiration air or non-purulent secretion was ...ned from 7 patients (group 1) and purulent secretion ...2 patients (group 2)

	IgA (mg/ml)	IgG (mg/ml)	IgM (mg/ml)
...up 1			
...etion			
M±S D	2 49±0 91	9 20±2 78	0 79±0 35
Range	1 34–3 81	5 66–13 84	0 24–1 22
...um			
M±S D	3 12±0 72	13 28±1 09	1 11±0 44
Range	2 61–4 20	11 17–14 81	0 62–1 93
...oup 2			
...cretion			
M±S D	0 96±0 67	4 36±4 36	0 58±0 38
Range	0 18–2 22	0 33–13 71	<0 27*–1 47
...um			
M±S D	2 63±1 13	14 24±4 21	1 34±0 51
Range	1 12–5 45	7 98–23 86	0 56–2 49

...Not demonstrable in two samples

IgA IgG and IgM were determined in sinus secretion ...rated prior to drainage and in serum from all patients ...e immunoglobulins, C3, C4, α_2-macroglobulin and al...min were determined in 16 of these patients not only ...erum and in sinus secretion obtained prior to drainage ... also in the secretions obtained 1–2 days after the ...inage The proteolytic activity was measured in the ...ulent secretions from 12 of the patients From another ...patients, sinus secretion of serous type was aspirated ...the analysis of proteolytic activity

Quantification of protein

The stored secretions were diluted 1 3 in phosphate-buffered saline (pH 7 4) and homogenized by gentle shaking The supernatant, after centrifugation at 3 000 rpm for 15 minutes, was used for protein determination The concentrations of IgA, IgG, IgM, albumin, α_2 macroglobulin and the complement factors C3 and C4 in serum and diluted secretion were determined by the single radial immunodiffusion method essentially as described by Mancini et al (1965) The mean normal serum value by the method used was for IgA 1 97±0 65 mg/ml, for IgG 11 18±2 0 mg/ml and for IgM 0 74±0 24 mg/ml C3 C4 and α_2 mac... bulin were expressed as a percentage of a normal ser... ...ool (normal serum range 60–140 %, 40–200 % and 50– respectively. The lowest value determinable by the method using diluted secretion was for IgA 0 12, IgG 0 27 and IgM 0 27 mg/ml and for α_2-macroglobulin C3 and C4 9 3 and 3 %, respectively A serum IgA was used as standard in all IgA determinations All analyses were performed in duplicate The relative analytic errors in the protein determinations varied between 4–7 % with the exception of α_2-macroglobulin in the secretion which was 12 %

Proteolytic activity

Fresh samples of maxillary sinus secretion were centrifuged at 3 000 rpm for 15 minutes after dilution (1 4) in phosphate buffered saline The supernatants were stored at −20°C The activity was determined by a modification of the method described by Kunitz (1947), using heat denaturated casein (Nutritional Biochemicals Corp , [illegible]

supernatant was read at 280 nm against a reaction blank for each sample One unit was defined as an increase of 1 0 in the absorbance at 280 nm

...ble II ***Immunoglobulins (mg/ml), complement and α_2-macroglobulin (α_2M) (% of a normal ...um pool) in serum and in purulent sinus secretion from 16 patients prior to drainage (Sample ...nd after drainage (sample II)***

	IgA (mg/ml)	IgG (mg/ml)	IgM (mg/ml)	C3 (%)	C4 (%)	α_2 M (%)
...retion						
...ple I						
...±S D	0 96±0 71	4 94±4 82	0 67±0 40	35±35	24±21	40±30
...ange	0 18–2 22	0 33–13 71	<0 27ᵇ–1 47	<3ᶜ–116	<1ᶜ–[illegible]	<9ᵃ–90
...ple II						
...±S D	1 90±1 35	8 76±6 86	0 90±0 58	56±52	38±31	62±44
...ange	0 44–5 55	0 86–20 43	<0 27ᵃ–2 19	<3ᵃ–184	<3ᵃ–91	9–143
...m						
...±S D	2 67±1 04	14 39±4 42	1 43±0 52	133±32	119±37	[illegible]
...ange	1 12–4 88	7 98–23 86	0 56–2 49	61–180	52–209	

...-demonstrable levels in ᵃone sample ᵇ2–3 [illegible]

Table I *Results of psychosomatic investigations in pollenosis and vasomotor rhinitis*

	Number of subjects	Neuroticity scoring (arithmetic means)		Neuroses[b]	Exacerbated by mental stress[b]
		Males	Females		
Pollenosis	23	−16 83 (−10 1)[a]	−9 7 (−4 3)[a]	8 7	17 3
Vasomotor rhinitis	28	+ 3 75 (−10 1)[a]	+9 56 (−4 3)[a]	32 1	67 9

[a] Controls—Warsaw general population (Bizon 1975) [b] Percentage of cases

treatment in just one female patient, in whom there was also overt neurosis

Slightly more often improvement was recorded in vasomotor rhinitis, although it was usually temporary and occurred at the beginning of treatment Interestingly, even exacerbation was seen in 2 patients

Phonostimulation, our second method, produced even less of a therapeutic effect

Our results show both methods to be ineffectual in rhinitis Some slight and transient improvements in the vasomotor kind should rather be attributed to suggestion, which, incidentally, must have been stronger in acupuncture The placebo type effect, observed in various kinds of treatment, is known to be stronger, the greater the role of psychic fac
s in the etiopathogenesis of certain somatic
r' This explains why the effects of
method on the symptoms were as good
nil in pollenosis Suggestion need not al
ys tend to mitigate the symptoms of a disease, quite the contrary, it may exacerbate them For instance, when the patient has r faith in the treatment and is negatively di posed toward it, even without realising this I a few cases of vasomotor rhinitis there w this kind of adverse response in the course treatment The presumably underlying nega tive attitude towards the treatment may hav been due to various factors, notably distru of its efficacy, as well as a sense of disappoin ment at receiving it not from a physician co trary to the expectations of some patients a least, but from a nurse, even though a spec ally trained one

CONCLUSIONS

1 Acupuncture and phonostimulation wer therapeutically ineffective in pollenosis

2 In vasomotor rhinitis the two method somewhat mitigated the symptoms temporari ly in certain of the patients and exacerbate them in a few We believe these effects to b attributable to suggestion rather than any kin

Table II *Effects of acupuncture and phonostimulation in vasomotor rhinitis and pollenosis*

	Acupuncture					Phonostimulation				
	Number of subjects	Relieved	Improved	Unchanged	Exacerbated	Number of subjects	Relieved	Improved	Unchanged	Exacerbated
First week										
Vasomotor rhinitis	14	1	4	7	2	14	–	2	9	3
Pollenosis	8	–	1	7	–	15	–	1	13	1
After full course										
Vasomotor rhinitis	14	–	2	11	1	14	–	1	12	1
Pollenosis	8	–	–	8	–	15	–	1	14	–

"specific" action either of acupuncture or phonostimulation

ZUSAMMENFASSUNG

…undzwanzig Patienten mit vasomotorischer Rhinitis … dreiundzwanzig mit Heuschnupfen wurden psychologisch untersucht und ausschließlich entweder durch Akupunktur oder Phonostimulation behandelt Die erste …rde in 22 Fallen der klassischen … de nach ange…ndet und die zweite in 29 Falle Die Ergebnisse …den auf Grund laryngologischer U…uchungen und …den Patienten gefuhrten besondere …htungstage …hern beurteilt Beide Methoden bl… ohne Ein… auf den Verlauf von Heuschnupfen In Rhinitis vasomotorica dagegen deren Verlauf ps…he Faktoren beeinflußten beobachtete man … Teil der Kranken gewöhnlich eine zeitweilige B… ung und in … paar Fallen eine Verscharfung der Symp… me Beides … auf Suggestion zuruckzufuhren sein

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	Acupuncture Number of subjects	Relieved	Improved	Unchanged	Exacerbated	Phonostimulation Number of subjects	Relieved	Improved	Unchanged	Exacerbated
First week										
Vasomotor rhinitis	14	1	4	7	2	14	–	2	9	3
Pollenosis	8	–	1	7	–	15	–	1	13	1
After full course										
Vasomotor rhinitis	14	–	2	11	1	14	–	1	12	
Pollenosis	8	–	–	8	–	15	–	1	14	

specific' action either of acupuncture or iostimulation

ZUSAMMENFASSUNG

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MAXILLARY SINUSITIS

Effects of Treatment on the Local Antibacterial Defence

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(Received December 20 1976)

Abstracts Low levels of immunoglobulins and complement in purulent antral secretion might jeopardize the local antibacterial defence To evaluate whether or not antibiotic treatment and antral drainage influence the local content of immunoglobulins and complement samples of secretion were analysed both prior to drainage and 1–2 days after drainage In those patients requiring repeated drainage for their recovery, the local concentration of IgA and IgG was significantly lower in the secretion obtained prior to drainage than the local concentration found in patients who recovered after the initial drainage It was also demonstrated that after drainage the level of IgA IgG and the complement factors C3 and C4 increased significantly whereas the proteolytic activity in the secretion usually decreased

Virulent microorganisms have not only the capacity to replicate in the biochemically complex environment of the host surface, fluid and tissue, but also the ability to resist the host defence, of which immunoglobulins and complement are important components

In a previous study, immunoglobulins were demonstrated in maxillary sinus secretion, the concentration being lower in purulent than in serous secretions (Carenfelt et al , 1976) As pus and certain purulent secretions have a high proteolytic activity (Lieberman & Kurnick, 1962) and as immunoglobulins and complement are susceptible to degradation by proteases, it was surmised that low levels of immunoglobulins and complement in purulent sinus secretion might result from proteolytic degradation

In the present study, the immunoglobulins IgA, IgG and IgM, the complement factors C3 and C4, and the protease inhibitor α_2 macroglobulin were determined in purulent sinus secretion in order to evaluate whether the treatment (including drainage of the purulent secretion) might influence the content of these components in the sinus secretion

This study was supported by grants from Sigurd and Elsa Golje Memorial Foundation

MATERIAL AND METHODS

Patients

Twenty nine patients (21 female and 8 male age range 22–65 years) with symptoms of maxillary sinusitis for 10 days or less and with a retained purulent secretion in *sinus maxillaris* were selected for the present study A *sample of sinus secretion (0 5–1 ml) was obtained* by aspiration through a Lichtwitz cannula inserted into the antrum through the inferior nasal meatus The remaining secretion was then evacuated as completely as possible resulting in volumes varying between 1 and 12 ml The antral drainage was repeated at intervals of 1–2 days until the patient had recovered that is until air or a minor volume (<1 ml) of non purulent secretion was obtained Irrigation was not used The therapy also included penicillin V or doxycycline orally administered in ordinary dosis together with decongestive drugs The antibiotic treatment was usually initiated after the first drainage

Samples of secretion macroscopically tinged with blood were discarded excepting those samples obtained on the second or third aspiration (5 of 16 secretions) Samples of venous blood for serum were taken simultaneously The secretion samples and sera were stored at −20°C until analysed immunologically

ble I *Mean immunoglobulin concentration* *(g/ml) in serum and purulent sinus secretion* *ained prior to drainage from 29 patients*

Repeated aspiration air or non purulent secretion was ined from 7 patients (group 1) and purulent secretion 22 patients (group 2)

	IgA (mg/ml)	IgG (mg/ml)	IgM (mg/ml)
p 1			
etion			
±S D	2 49±0 91	9 20±2 78	0 79±0 35
nge	1 34–3 81	5 66–13 84	0 24–1 22
m			
±S D	3 12±0 72	13 28±1 09	1 11±0 44
nge	2 61–4 20	11 17–14 81	0 62–1 93
p 2			
etion			
±S D	0 96±0 67	4 36±4 36	0 58±0 38
nge	0 18–2 22	0 33–13 71	<0 27*–1 47
n			
±S D	2 63±1 13	14 24±4 21	1 34±0 51
nge	1 12–5 45	7 98–23 86	0 56–2 49

t demonstrable in two samples

IgG and IgM were determined in sinus secretion ited prior to drainage and in serum from all patients mmunoglobulins C3 C4 α_2-macroglobulin and al were determined in 16 of these patients not only um and in sinus secretion obtained prior to drainage lso in the secretions obtained 1–2 days after the ige The proteolytic activity was measured in the ent secretions from 12 of the patients From another tients sinus secretion of serous type was aspirated e analysis of proteolytic activity

Quantificati n of protein

The stored secretions were di ted 1 3 phosph te buffered saline (pH 7 4) and h mogenize by gentle shaking The supernatant after ce ntrifugatio at 3 000 rpm for 15 minutes was used for pr n deter ina on [illegible] in α_2 m ro- [illegible] l C4 in se um [illegible] ngle rac [illegible] by Mar cini et al (1965) The mean normal serum v hy the method used was for IgA 1 97±0 65 mg/ml IgG 11 18±2 06 mg/ml and for IgM 0 74±0 24 mg/ml C4 and α_2-macroglobulin were expressed as a percentage of a normal serum pool (normal serum range 60–140% 40–200% and 50–150% respectively The lowest value determinable by the method using diluted secretion was for IgA 0 12 IgG 0 27 and IgM 0 27 mg/ml and for α_2-macroglobulin C3 and C4 9 3 and 3% respectively A serum IgA was used as standard in all IgA determinations All analyses were performed in duplicate The relative analytic errors in the protein determinations varied between 4–7% with the exception of α_2-macroglobulin in the secretion which was 12%

Proteolytic activity

Fresh samples of maxillary sinus secretion were centrifuged at 3 000 rpm for 15 minutes after dilution (1 4) in phosphate buffered saline The supernatants were stored at −20°C The activity was determined by a modification of the method described by Kunitz (1947) using heat denaturated casein (Nutritional Biochemicals Corp Cleveland Ohio) as the substrate The reaction mixture (4 2 ml) consisted of 1% (w/v) casein 0 025 M potassium phosphate pH 7 4 0 5 mM Ca^{++} and 0 2 ml of the sample After incubation for 30 minutes at 37°C the reaction was terminated by the addition of 3 ml of 10% (v/v) per chlorid acid After centrifugation the absorbance of the supernatant was read at 280 nm against a reaction blank for each sample One unit was defined as an increase of 1 0 in the absorbance at 280 nm

le II *Immunoglobulins (mg/ml), complement and α_2-macroglobulin (α_2-M) (% of a normal m pool) in serum and in purulent sinus secretion from 16 patients prior to drainage (Sample d after drainage (sample II)*

	IgA (mg/ml)	IgG (mg/ml)	IgM (mg/ml)	C3 (%)	C4 (%)	α_2-M (%)
tion						
le I						
±S D	0 96±0 71	4 94±4 82	0 67±0 40	35±35	24±23	40±30
ige	0 18–2 22	0 33–13 71	<0 27*–1 47	<3c–116	<3c–63	<9*–90
le II						
±S D	1 90±1 35	8 76±6 86	0 90±0 58	56±52	38±31	6*
ige	0 44–5 55	0 86–20 43	<0 27*–2 19	<3b–184	<3b–95	
n						
±S D	2 67±1 04	14 39±4 42	1 43±0 52	133±32	119±37	
ige	1 12–4 88	7 98–23 86	0 56–2 49	61–180	[illegible]	

demonstrable levels in * one sample b 2–3 samples c 5–6 samples

Table III *The ratios between protein contents in sinus secretions aspirated prior to drainage (I) after the first (II) and second (III) drainage*

	Ratio II/I (n=16)		Ratio III/I (n=5)	
	Mean	Range	Mean	Range
IgA	2 4	1 1– 4 8	3 7	1 3–10 7
IgG	2 7	1 2– 8 1	6 0	1 2–14 2
IgM	1 7	0 6– 6 5	2 3	1 1– 3 9
C3	3 0	0 9–12 9	4 8	1 2–11 7
C4	2 6	0 9–>9 3	5 8	1 1–16 8
α_2-M	1 8	1 0–>2 6	3 7	1 2–11 7
Alb	2 9	1 1– 8 1	5 5	1 1–17 4

Statistical methods

The relative errors were calculated according to the for mula

$$\sqrt{\frac{\sum\left(\frac{d}{x}\right)^2}{2n}}$$

where d is the difference between the two determinations x is the mean value of the two determinat ons and n is the number of duplicate determinations For significance ana lysis the Wilcoxon rank sum test was used

RESULTS

The content of the immunoglobulins, the com plement factors and α_2-macroglobulin in serum was usually within the normal range The con ... on of IgA, IgG and IgM determined in ... antral secretions from 29 patients ... ses) prior to drainage is shown in Table ... of these cases air or non purulent se

Table IV *Bacterial isolate and outcome in 6 patients with low levels of C3 and C4 in the sinus secretion (per cent of normal serum pool)*

Sample I prior to drainage sample II after drainage

Case no	Bacterial isolate	Sample I C3	Sample I C4	Sample II C3	Sample II C4	Outcome
1	Pneumoc	<3	<3	27	28	Cured
2	Pneumoc	9	<3	56	12	Cured
3	Anaerobes	<3	<3	<3	<3	Not cured
4	Anaerobes	<3	<3	9	<3	Not cured
5	Anaerobes	<3	<3	4	8	Cured
6	Anaerobes	<3	<3	<3	<3	Cured

Table V *Proteolytic activity in purulent ... serous sinus secretion (units/ml)*

Sample I prior to drainage sample II after drainage

	Purulent		Serous
	Sample I	Sample II	Sample I
Mean	3 23	1 78	0 22
S D	3 63	1 68	0 28
Range	0–9 92	0–4 72	0–0 74
n	12	7	10

cretion was obtained on aspiration 1–2 da... later In 22 cases, purulent secretion was s... present on the second aspiration In the gro... of patients (group II in Table I) requiring ... peated drainage for recovery, as defined in th... study, the mean concentration of IgA and Ig... was lower than the corresponding values ... the group of patients requiring only a sing... drainage for recovery (p<0 01)

Samples of purulent sinus secretion o... tained prior to drainage and 1–2 days aft... drainage, were analysed in 16 of the patien... (16 sinuses) As shown in Table II, the secr... tion proteins increased after drainage (p... 0 01) The increase in IgM, however, was n... significant The ratios between values ob... tained at the second and at the first sampli... show a 1 7- to 3 0-fold increase of the secr... tion proteins (Table III) A further increase ... the protein content in the secretion was r... corded in the third sample obtained 1–2 day... after the second drainage (Table III)

No demonstrable α_2-macroglobulin w... found in 3 out of 16 samples obtained prior t... drainage, but at the second sampling the leve... had increased to 25% (average) of the mea... serum value Furthermore, no complement c... barely measurable secretion levels were foun... in 6 of 16 patients (Table IV) After drainage... the level of complement increased in 2 case... from which pneumococci in pure culture wer... isolated In the remaining 4 cases from whic... anaerobic bacteria were isolated (*Peptostrep... tococcus anaerobius* and *Streptococcus inter... medius*), no or only moderate increase in com plement was demonstrable in the secretions

wo of these patients were not cured by the therapy used

Proteolytic activity was determined in purulent sinus secretion obtained from 12 patients prior to drainage as well as secretions obtained from 7 of these patients 1–2 days after draining (Table V) After drainage, the proteolytic activity decreased in 5, increased in 1, and remained undemonstrable in 1 secretion For comparison, the proteolytic activity was determined in 10 sinus secretion samples of serous type taken prior to drainage No activity was recorded in 5 of these and in the remainder a low activity was observed The proteolytic activity did not appear to be related to any particular bacterial species isolated in the secretion cultures

DISCUSSION

Of several antibacterial mechanisms mediated by the immunoglobulins, whether transferred from serum or locally produced, the role of local immunity and the role of IgA have been particularly in focus Thus, Fubara & Freter (1972) and Williams & Gibbons (1972) demonstrated that locally synthesized antibodies of the IgA type (S-IgA) protected mucosal surfaces from bacterial attachment and colonization This finding also contributes to the understanding of the pathogenesis of bacterial infections of mucous membranes Another suggested antibacterial mechanism, mediated by IgA independently of complement, is the ability of the antibody to promote phagocytosis (Girard & de Kalbermatten, 1970, Wernet et al , 1971, Spiegelberg et al , 1974) It has further been shown that antibodies, at least those of IgG class and complement, enhance the intragranulocytic extermination of certain bacteria (Christie et al , 1976, Solberg et al , 1976) As the content of immunoglobulins is lower in purulent than in serous sinus secretion (Carenfelt et al , 1976) and as a high proteolytic activity in purulent secretion might cause degradation of immunoglobulins and complement factors, it was questioned whether drainage of the antral secretion influences the protein levels in the secretion

The results obtained by the immunodiffusion method are connected with certain errors In purulent sinus secretion the presence of immunoglobulin fragments of heterogenic molecular size must be expected due to proteolytic degradation These fragments might influence the values obtained in such a way that the secretion values might be overestimated Thus the protein concentration measured can be considered to be representative of the maximum possible amount of the immunoglobulin present (Tomasi & Bienenstock, 1968) Multiple precipitation zones were rare

When aspirating sinus secretion, an undue admixture of blood could not always be avoided However, samples macroscopically tinged with blood obtained at the first aspiration were discarded The Guaiac test was not utilized, as the remaining samples could be assumed to contain negligible amounts of blood (Biberfeld & Sterner, 1971) On the other hand, in the second and third sets of aspiration, secretion samples tinged with blood were accepted as the composition of these secretions together with an admixture of serum products reflects the antral environment obtained on the drainage procedure But most of these samples were not tinged with blood

Only 16 of 22 sinus secretions obtained after drainage were included in the study of the treatment effects on local immunity The remainder were discarded either because of too small secretion volumes or because of high viscosity of the secretions which made a proper management impossible

It is interesting to note that the present material shows a relationship between the concentration of immunoglobulins in the secretion and the recovery from maxillary sinusitis, in that patients who recovered after the first drainage had a higher mean concentration of IgA and IgG in the secretion prior to drainage than those patients who did not This d[illegible] ence in immunoglobulin concentrat[illegible]

[illegible] as significant It is also ev[illegible]

antral content of IgA, IgG, C3 and C4 increased on average 2–3 times after one drainage, an increase that seemed to be augmented by repeated drainage Despite the possibility of a certain overestimation of the protein levels in the purulent secretion due to the immunodiffusion method used, the conclusions are not jeopardized Such an overestimation is in all probability related mostly to those secretion samples taken prior to drainage, as their exposure to proteolytic enzymes was longer than that of samples taken later

Although a certain contribution of the content of immunoglobulins found in the secretion may have a local source (Carenfelt et al, 1976), an increased transudation of serum products following the drainage might explain the increase in secretion proteins This is indicated by the fact that albumin increased more than proteins of higher molecular weight, but also by the fact that the increase in proteins was essentially the same when only secretions without admixture of blood were considered

While C4 together with antibodies takes part in the classical mode of complement activation, C3 is also involved in the activation of complement by the alternate pathway (Gotze & Muller Eberhard, 1971) and is the plement factor of major importance for ocyte adherence, phagocytosis and re of lysosomal constituents (Henson, The alternate route of C3 activation is e basis of complement mediated opsonization prior to the antibody response Certain bacteria, such as pneumococci of various types (Winkelstein et al 1976) activate the alternate pathway which appears to be of importance in the opsonization of pneumococci (Forsgren & Quie 1974) In patients with a serum deficiency of C3 or its proactivator, reduced phagocytosis and increased susceptibility to bacteria such as pneumococci, is found (Alper et al 1973 Forsgren, 1975) The low level of C3 found in most sinus secretions is therefore of special interest as a local deficiency of C3 may then seriously handicap local resistance to pneumococci and other species This is also supported by t fact that 2 of these 4 patients in the prese study who had extremely low levels of C3 secretion before as well as after drainage we not cured by the therapy

Immunoglobulins are susceptible to c gradation in vitro by proteolytic enzymes su as trypsin and chymotrypsin, although S I to a lesser extent (Brown et al, 1970 Linc 1975) As proteolytic enzymes are releas from frustrated granulocytes or in connecti with the death of the cells, and since hum granulocytes contain chymotrypsin like e zymes (Odeberg et al, 1975) a degradation immunoglobulins to fragments is to be e pected in pus Such split products of immun globulins have been demonstrated in v (Kastenbauer et al, 1975) Since the granul cyte proteases also have the capacity to sp complement factors (Janoff, 1972) proteolyt degradation may explain the low levels of in munoglobulins and complement found in tl purulent sinus secretion This concept is su ported by findings of high proteolytic activi in most of the purulent sinus secretions in co trast to serous secretions containing a cor paratively small number of inflammatory cel (Carenfelt & Lundberg, 1977), and also by th low level of the protease inhibitor α_2-macr globulin found in some of the secretions W expected to find a relationship between th length of the history of sinusitis and the cor tent of immunoglobulins and complement i the secretion Apart from the fact that thos 2 patients who were not cured (Table IV) ha suffered from purulent nasal discharge for or month, no such relationship was apparen probably because of the difficulty in establisl ing the true duration of the sinusitis

Thus the present results indicate a rel tionship between the content of immunoglobu lins and the complement in the purulent sinu secretion and the recovery from the sinusitis The results also provide evidenc treatment of the maxillary sinus comprising not only antibiotics bu peated drainage of the retained pu

on, increases the content of immuno ulins and complement locally at the site of nfection, and thus improves the prerequi- for the antibacterial defence to operate uately

ACKNOWLEDGEMENTS

ncere thanks are extended to Mrs Katarina Karlen ilful technical assistance

ZUSAMMENFASSUNG

ige Spiegel von Immunglobulinen und Komplement ngem Sekret des Sinus maxillaris konnten die ört antibakterielle Abwehr beeinträchtigen Um festzu a ob antibiotische Behandlung und Abzug aus der rhöhle den örtlichen Gehalt an Immunglobulinen und plement beeinflussen wurden Proben von Sekret siert und zwar vor dem Abzug ebenso wie 1–2 Tage

r aufgezeigt daß nach einem Abzug der Spiegel von und IgG sowie der Komplementfaktoren C3 und C4 ikant zunahm während die proteolytische Aktivität ekret zumeist abnahm

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ACUPUNCTURE AND PHONOSTIMULATION IN POLLENOSIS AND VASOMOTOR RHINITIS IN THE LIGHT OF PSYCHOSOMATIC INVESTIGATIONS

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(Received January 17, 1977)

Abstract Patients with vasomotor rhinitis (28) and pollenosis (23) were subjected to psychosomatic examination and treated either by acupuncture or phonostimulation exclusively Acupuncture was performed after the classical method in 22 patients and phonostimulation in 29 Evaluation of the results was based on laryngological examinations and appraisals entered by the patients in special personal diaries In pollenosis the condition was unchanged by the treatment In vasomotor rhinitis on the other hand in which psychic factors were of importance some of the patients usually improved temporarily at the beginning of treatment whereas a few suffered exacerbation These effects may be attributable to suggestion

Acupuncture, a method of treatment with a ꞉ of 3 000 years in Chinese medicine and insertion of needles to a depth of up cm at strictly defined points of the body, in the past few decades aroused interest both in Europe and the United States (Mann, 1971) In modern time it has been used as an analgesic method (Dimond 1971, Mann, 1972, Borzęcki & Borzęcki 1975) as well as in the treatment of certain chronic diseases It is reported to have produced very good results in vasomotor rhinitis (Su In 1958 Mann, 1971) hay fever (Mann, 1971) and bronchial asthma (Su In, 1958 Vogalik, 1959 Mann, 1971) It has been particularly efficacious in the treatment of Meniere's syndrome (Su In 1958, Mann, 1971)

This project was supported by the Central Institute of Mining

The mechanism operative in the method is not well understood In efforts to explain its analgesic effects, reference has also been made to the concept of Head s zones of hyperalgesia—areas of cutaneous sensitivity corresponding to a metameric distribution of the innervation of viscera As applied to hypersensitivity diseases it seems best explained as stimulation of the amygdaloid nucleus, which is part of the limbic system (Mandell et al, 1963) Specific stimulation of skin areas is said to excite the amygdala, which then modifies the levels of steroid hormones in the blood via the hypothalamus-pituitary-adrenal axis In this regard, Boeva and colleagues (1963) demonstrated that "stimulation acupuncture" produced an increase in the endogenous production of ACTH, and subsequently in adrenocortical hormones

Unlike acupuncture, phonostimulation is a new method, used in our Centre for Analgesia for the control of chronic pain It acts on the teleceptors of the organ of hearing The mechanism of its analgesic action involves the CNS but is also poorly understood

The poor effects of various treatments in rhinitis, especially those of the vasomotor type, have prompted us to try physical methods, which, if effective, would have the added but vastly important advantage of being completely non toxic

MATERIAL AND METHOD

he investigations were made in 51 random-lected patients with rhinitis, 28 of them with e vasomotor type and 23 with pollenosis he sex ratio was 32 females to 19 males The e range was 18–60 years, the average being 9 years and 37 1 for patients with pollenosis d vasomotor rhinitis respectively, for which e respective mean durations were 5 and 5 9 ars

Psychosomatic examination was performed each case as described elsewhere (Czubal-i & Zawisza, 1976, Czubalski et al , 1976) ith the neuroticity level determined according to the scale of Z Bizon (1975) The results f psychosomatic investigations were to indiate (1) the role of psychic factors in pollenos and vasomotor rhinitis, and (2) the relaon between that role and the effects of treat ent

Acupuncture was performed according to e classical principles Disposable hypoderic needles were inserted to a depth of 1 5–2 0 m at two symmetrical points between the 1st nd 2nd epiphyses of the metacarpal bones Ho-Ku points), and to a depth of 3 mm at wo symmetrical points to the sides of the asal alae (Hei Liao points) The needles ere left inserted for 15 minutes and slightly otated with the fingers every 5 minutes The rocedure was performed in all patients by the ame person, a specially trained nurse A full ourse involved 12 such treatments two per week, and was given to 14 patients with vasomotor rhinitis and 8 with pollenosis

In the second method phonostimulation the patient received a pair of headphones and

at the frequency of 1 5 Hz was used in 14 cases of vasomotor rhinitis and 15 of pollenosis

In order to estimate the effects of treatments we considered (*a*) the pain threshold, which was usually higher after a treatment (measured in the leg by the mechanical method), (*b*) skin temperature of the face, measured before and after treatments with an "Aga" instrument, and, in a part of the patients, (*c*) EEG records taken before, during, and after treatments, the change they usually showed was the pattern of the first stage of sleep

To evaluate the ultimate results, laryngological examinations were made in each case three times at the start of a course, at the beginning of the second week, and after the full course In addition, the patients themselves judged the results of treatment in a special diary they had to keep In evaluations of the method four classical symptoms of the disease were considered blocking of the nasal passages, watery discharge, sneezing, and itching

RESULTS AND DISCUSSION

The results are recorded in Tables I and II As can be seen in Table I, psychic factors are important in vasomotor rhinitis, exacerbating the symptoms in almost two-thirds of the pa tients The neuroticity level, as measured on the neuroticity scales, was distinctly higher in this disorder, making even overt neurosis a frequent finding (32 1%) This cannot be ex plained very well by the long standing of the complaint (5 9 years on average) and its usually distressing character, because in pollenosis, which is similarly trying, overt neurosis was rare and neuroticity was even below the level recorded in the general population in Po land (Bizon, 1975), although the average dura tion of the condition (5 years) was not very different Psychic factors played no significant role in pollenosis, just as recorded earlier in a different material (Czubalski & Zawisza 1976)

In contrast to reports by some authors (Su In, 1958, Vogralik, 1959, Boeva et al , 1963, Mann, 1971), acupuncture was found ineffec tive, both in vasomotor rhinitis and in pollenosis (Table II) In the latter we achie therapeutic effects at all except and transient improvement at

Table I *Results of psychosomatic investigations in pollenosis and vasomotor rhinitis*

	Number of subjects	Neuroticity scoring (arithmetic means) Males	Females	Neuroses[b]	Exacerbated by mental stress[b]
Pollenosis	23	−16 83 (−10 1)[a]	−9 7 (−4 3)[a]	8 7	17 3
Vasomotor rhinitis	28	+ 3 75 (−10 1)[a]	+9 56 (−4 3)[a]	32 1	67 9

[a] Controls—Warsaw general population (Bizoń 1975) [b] Percentage of cases

treatment in just one female patient, in whom there was also overt neurosis

Slightly more often improvement was recorded in vasomotor rhinitis, although it was usually temporary and occurred at the beginning of treatment Interestingly, even exacerbation was seen in 2 patients

Phonostimulation, our second method, produced even less of a therapeutic effect

Our results show both methods to be ineffectual in rhinitis Some slight and transient improvements in the vasomotor kind should rather be attributed to suggestion, which, incidentally, must have been stronger in acupuncture The placebo type effect, observed in various kinds of treatment, is known to be stronger, the greater the role of psychic factors in the etiopathogenesis of certain somatic [illegible] This explains why the effects of [illegible] d on the symptoms were as good [illegible] nil in pollenosis Suggestion need not always tend to mitigate the symptoms of a disease, quite the contrary, it may exacerbate them For instance, when the patient has no faith in the treatment and is negatively disposed toward it, even without realising this In a few cases of vasomotor rhinitis there was this kind of adverse response in the course of treatment The presumably underlying negative attitude towards the treatment may have been due to various factors, notably distrust of its efficacy, as well as a sense of disappointment at receiving it not from a physician, contrary to the expectations of some patients at least, but from a nurse, even though a specially trained one

CONCLUSIONS

1 Acupuncture and phonostimulation were therapeutically ineffective in pollenosis

2 In vasomotor rhinitis the two methods somewhat mitigated the symptoms temporarily in certain of the patients and exacerbated them in a few We believe these effects to be attributable to suggestion rather than any kind

Table II *Effects of acupuncture and phonostimulation in vasomotor rhinitis and pollenosis*

	Acupuncture					Phonostimulation				
	Number of subjects	Relieved	Improved	Unchanged	Exacerbated	Number of subjects	Relieved	Improved	Unchanged	Exacerbated
First week										
Vasomotor rhinitis	14	1	4	7	2	14	–	2	9	3
Pollenosis	8	–	1	7	–	15	–	1	13	1
After full course										
Vasomotor rhinitis	14	–	2	11	1	14	–	1	12	1
Pollenosis	8	–	–	8	–	15	–	1	14	–

of "specific" action either of acupuncture or phonostimulation

ZUSAMMENFASSUNG

Achtundzwanzig Patienten mit vasomotorischer Rhinitis und dreiundzwanzig mit Heuschnupfen wurden psychosozial untersucht und ausschließlich entweder durch Akupunktur oder Phonostimulation behandelt Die erste wurde in 22 Fallen der klassischen Methode nach angewendet und die zweite in 29 Fallen Die Ergebnisse wurden auf Grund laryngologischer Untersuchungen und

[illegible]

motorica dagegen deren Verlauf psychische Faktoren beeinflußten beobachtete man in einem Teil der Kranken gewöhnlich eine zeitweilige Besserung und in ein paar Fallen eine Verscharfung der Symptome Beides mag auf Suggestion zuruckzufuhren sein

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Pollenosis	8	–	–	8	–	15	–	1	14	–

'pecıfic" actıon eıther of acupuncture or
oostımulatıon

ZUSAMMENFASSUNG

undzwanzıg Patıenten mıt vasomotorısc e Rhınıtıs dreıundzwanzıg mıt Heuschnupfen wurd n psychoısch untersucht und ausschließlıch entweder durch punktur oder Phonostımulatıon behardelt D e erste e ın 22 Fällen der klassıschen Methode nach anre det und dıe zweıte ın 29 Fällen D e Ergebnısse den auf Grund laryngologıscher Untersu hungen und den Patıenten geführten besonderen Gu ıchtungstage hem beurteılt Beıde Methoden blıeb n ohne E n auf den Verlauf von Heuschnupfen In Rhınıtıs va otonıca dagegen deren Verlauf psychısche Fakto beeınflußten beobachtete man ın e nem Teıl der nken gewohnlıch eıne zeıtweılıge Be se ung und ın paar Fällen eıne Verschärfung der Symptome Beıdes auf Suggestıon zuruckzuführen seın

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A DENTAL SPLINT FOR USE DURING PERORAL ENDOSCOPY

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(Received April 1 1977)

Abstract Damage to teeth is a well known complication of peroral endoscopy A case is described in which potentially fatal complications of such damage occurred This spurred on the development of a dental splint as an aid in the prevention of such damage This splint is described and evidence for the beneficial results of its use is presented

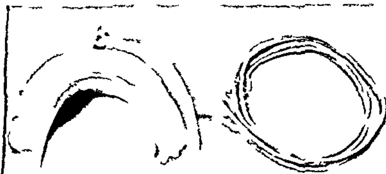
It has been known for many years that peroral endoscopy, for whatever indication, can result in damage to the teeth, which damage can have potentially lethal consequences Even if not life threatening damage to the incisor, for example, is an additional imposition on an in ' who may already be seriously ill

nus a 68 year old man was admitted to an clinic for operative treatment of osteoarthritis of the hip After the operation, which was performed under general anaesthesia with endotracheal intubation it was noticed that two teeth were missing One was immediately recovered and a chest X-ray revealed that the other had lodged in the patient s left lung The patient was moved to the department of laryngology where an attempt was made to remove the tooth by bronchoscopy A prolonged attempt lasting 2½ hours was unsuccessful Towards the end of the investigation the patient regurgitated and aspirated stomach contents tracheostomy was then performed Ventilation of the patient proved extremely difficult, and the cir began to fail It was apparent that this was to a left sided pneumothorax, presumably lowing damage to a bronchus and an costal drain was immediately inserted The tient was then moved to the thoracic dep ment for operative removal of the tooth sided thoracotomy was performed 2 later, and the missing tooth was removed bronchotomy The postoperative course complicated by an empyema which surgical drainage and antibiotic finally made a good recovery and left 2 months after his original admission

To avoid such catastrophes, a d splint has been developed in the Hospital in Stockholm It consists flexible plastic tray (Fig 1) non adhesive silicone base w' to the contours of the teeth This is covered by a plastic membrane its extrusion while in the mouth A tached to the splint to facilitate should it for any reason find its throat The splint is delivered in It should be placed (Figs 2 and 3) jaw, or on the lower jaw as well Should it be considered that the obstruct the view, it is easy to cut of it, using an ordinary pair of scis

The splint has now been in u of more than 6 years Prior to its we had approximately three or

The splint is manufactured by ASDI Box 420 S-19404 Upplands Väsby Sweden



F g 1 The spl nt nly part ally f'lled w th one base



F g 2 Spl nt covering upper teeth



Fig 3 I ess inst uments the teeth

dental damage associated with anaesthesia each year. Since its introduction we have had but one such case, when a short-term locum, eschewing its use, managed to knock out a tooth.

This favourable result has been attained in spite of the fact that during this period many uraemic patients and patients with hyperparathyroidism were anaesthesized. It is well known that both groups of patients are particularily liable to dental decay and parodontitis. Moreover, since Serafimer Hospital is a teaching hospital, many intubations are performed by medical students.

ZUSAMMENFASSUNG

Zahnverletzung ist eine erkannte Komplika·on für rale Endoskopie Ein Fall wird beschrieben wobei tuelle tödliche Komplikationen infolge einer dera Verletzung entstanden sind Dies hat zur Folge dass die Entwicklung von einer Zahnschiene, die sc Schäden vorbeugen kann, beschleunigt worden is schreibung der Schiene sowie Präsentation der Be fur die erfolgreichen Resultate

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Abstract Damage to teeth is a well known complicat on of peroral endoscopy A case is described in which potentially fatal complications of such damage occurred This spurred on the development of a dental splint as an aid in the prevention of such damage This spl nt is described and evidence for the beneficial results of its use is presented

It has been known for many years that peroral endoscopy, for whatever indication can result in damage to the teeth which damage can have potentially lethal consequences Even if not life threatening damage to the incisor, for example is an additional imposition on an in vidual who may already be seriously ill

us a 68 year old man was admitted to an opaedic clinic for operative treatment of steoarthritis of the hip After the operation which was performed under general anaesthesia with endotracheal intubation it was noticed that two teeth were missing One was immediately recovered and a chest X ray revealed that the other had lodged in the patient s left lung The patient was moved to the department of laryngology where an attempt was made to remove the tooth by bronchoscopy A prolonged attempt lasting 2½ hours was unsuccessful Towards the end of the investigation the patient regurgitated and aspirated stomach contents tracheostomy was then performed Ventilation of the patient proved extremely difficult and the circula began to fail It was apparent that this was to a left sided pneumothorax, presumably lowing damage to a bronchus and an int costal drain was immediately inserted The tient was then moved to the thoracic dep ment for operative removal of the tooth Le sided thoracotomy was performed 2 da later, and the missing tooth was removed aft bronchotomy The postoperative course w complicated by an empyema which requi surgical drainage and antibiotic therapy I finally made a good recovery and left hosp 2 months after his original admission

To avoid such catastrophes a disposabl splint has been developed in the Serafime Hospital in Stockholm It consists of a firm bu flexible plastic tray (Fig 1) containing a soft non adhesive silicone base which moulds itsel to the contours of the teeth This silicone mas is covered by a plastic membrane to preven its extrusion while in the mouth A cord is at tached to the splint to facilitate its recovery should it for any reason find its way into the throat The splint is delivered in a sterile pack It should be placed (Figs 2 and 3) on the upper jaw, or on the lower jaw as well if necessary Should it be considered that the splint might obstruct the view it is easy to cut away a part of it using an ordinary pair of scissors

The splint has now been in use for a period of more than 6 years Prior to its introduction we had approximately three or four cases of

The spl nt is manufactured by ASDI Box 470 S 19404 Upplands Väsby Sweden



Fig 1 The splint is only partially filled with silicone base



Fig 2 Splint covering upper teeth



Fig 3 Pressure from blunt instruments does not damage the teeth

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A DENTAL SPLINT FOR USE DURING PERORAL ENDOSCOPY

G McCarthy and O Carlson

From the Department of Anesthesia Serafimerlasarettet Stockholm Sweden

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It has been known for many years that peroral endoscopy, for whatever indication, can result in damage to the teeth, which damage can have potentially lethal consequences Even if not life threatening, damage to the incisor, for example, is an additional imposition on an individual who may already be seriously ill

Thus a 68 year-old man was admitted to an orthopaedic clinic for operative treatment of osteoarthritis of the hip After the operation, which was performed under general anaesthesia with endotracheal intubation, it was noticed that two teeth were missing One was immediately recovered and a chest X-ray revealed that the other had lodged in the patient s left lung The patient was moved to the department of laryngology where an attempt was made to remove the tooth by bronchoscopy A prolonged attempt lasting 2½ hours was unsuccessful Towards the end of the investigation, the patient regurgitated and aspirated stomach contents, tracheostomy was then performed Ventilation of the patient proved extremely difficult, and the circula began to fail It was apparent that this was to a left sided pneumothorax, presumably lowing damage to a bronchus, and an int costal drain was immediately inserted The tient was then moved to the thoracic depa ment for operative removal of the tooth L sided thoracotomy was performed 2 da later, and the missing tooth was removed aft bronchotomy The postoperative course w complicated by an empyema which requir surgical drainage and antibiotic therapy H finally made a good recovery and left hospit 2 months after his original admission

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dental damage associated with anaesthesia each year Since its introduction we have had but one such case, when a short term locum, eschewing its use, managed to knock out a tooth

This favourable result has been attained in spite of the fact that during this period many uraemic patients and patients with hyperparathyroidism were anaesthesized It is well known that both groups of patients are particularly liable to dental decay and parodontitis Moreover, since Serafimer Hospital is a teaching hospital, many intubations are performed by medical students

ZUSAMMENFASSUNG

Zahnverletzung ist eine erkannte Komplika on für per
rale Endoskopie Ein Fall wird beschrieben wobei pote
tielle tödliche Komplikationen infolge einer derartig
Verletzung entstanden sind Dies hat zur Folge geha
dass die Entwicklung von einer Zahnschiene die solch
Schäden vorbeugen kann beschleunigt worden ist B
schreibung der Schiene sowie Präsentation der Bewei
für die erfolgreichen Resultate

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SUBJECT INDEX